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**Role of maternal ethnicity, adverse risk
factors and gestational diabetes on offspring
body composition and adipocytokines**

by

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degree of
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Declaration

The current thesis is an original work and is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by myself and has not been submitted in any previous application for any degree.

Two papers, which have been created based on this research work, titled: i) Cord blood adipocytokines and body composition at birth and up to 5 years of age: A systematic review and ii) Impact of gestational diabetes mellitus on infant body composition, are currently under review for publication in medical journals.

Abstract

Childhood obesity has reached epidemic proportions globally. There is increasing evidence that factors acting during early development, such as maternal obesity, excessive gestational weight gain and gestational diabetes mellitus increase the chances of obesity and metabolic syndrome in later life. However, the major limitation of the available literature is the lack of accurate body composition measurement. In order to understand the mechanisms underpinning the developmental origins of metabolic diseases in adulthood, data on the evolution of body composition in early childhood is required. We hypothesize that offspring born to high metabolic risk mothers have altered body composition at birth and early infancy, which leads to adverse developmental programming of obesity and metabolic diseases.

Objective and methods: Purpose of the current research work was to study the effect of maternal adverse metabolic profile on infant's body composition. Secondary aim was to assess whether early life biomarkers can predict infant and early childhood adiposity, allowing the implementation of targeted prevention strategies on high risk groups from the early stages of life. A longitudinal observational study was performed. Air displacement plethysmography was used to assess infant body composition up to 5 months of age. A systematic review looking at the association between cord blood leptin, adiponectin and infant adiposity was also conducted.

Results: Maternal obesity, gestational weight gain and hyperglycaemia independently predict infant adiposity. Maternal insulin resistance seems to be the "missing link" between adverse maternal metabolic profile and neonatal adiposity. Current practices in the management of gestational diabetes that reduce the incidence of macrosomia happens at the expense of fat free mass. This may drive early adiposity rebound, which itself has shown to be an independent driver for metabolic diseases in adult life. Ethnic subgroup analysis revealed no difference in infant body composition, provided that maternal characteristics and intrauterine environment remain constant across ethnicity. Cord blood leptin is strongly and positively associated with fat mass at birth but inversely predicts adiposity up to 3 years of age.

Conclusion: Despite genotype being determined at conception, the phenotype is modulated and determined by maternal and intrauterine factors. Antenatal and early postnatal life are critical periods of developmental plasticity for offspring fat and fat free mass. Such adverse body composition at birth and early infancy are setting lifelong metabolic trajectories.

List of abbreviations

AC	Abdominal circumference	IADPSG	International Association of Diabetes and Pregnancy Study Groups
ADA	American Diabetes Association	IGF	Insulin like Growth factor
ADP	Air Displacement Plethysmography	IOM	Institute of Medicine
AGA	Appropriate for gestational age	IUGR	Intrauterine Growth Restriction
ARC	Arcuate Nucleus	LGA	Large for Gestational Age
BAT	Brown adipose tissue	LBW	Low Birth Weight
BF	Breastfeeding	MRI	Magnetic Resonance Imaging
BMI	Body Mass Index	NHS	National Health Service
BMR	Basic Metabolic rate	NICE	National Institute of Clinical Excellence
CNS	Central Nervous System	OGTT	Oral Glucose Tolerance Test
CS	Caesarian Section	OSA	Obstructive Sleep Apnoea
DXA	Dual energy X-ray absorptiometry	PCOS	Polycystic Ovarian Syndrome
EFW	Estimated Fetal Weight	RCT	Randomized Control Trial
ELISA	Enzyme-linked Immunosorbent Assay	RDS	Respiratory Distress Syndrome
FF	Formula Feeding	RIA	Radioimmunoassay
FFA	Free Fatty Acids	RR	Relative Risk
FFM	Fat Free Mass	SA	South Asians
FFMI	Fat Free Mass Index	SD	Shoulder Dystocia
FL	Femur Length	SGA	Small for gestational age
FM	Fat Mass	SS	Sub-scapular Skinfold
FMI	Fat Mass Index	SEE	Standard Error of Estimate
FPG	Fasting Plasma Glucose	TNF-a	Tumor Necrosis Factor a
GCT	Glucose Challenge Test	TR	Triceps
GDM	Gestational Diabetes Mellitus	TZD	Thiazolidinediones
GEH	George Eliot Hospital	UK	United Kingdom
GWG	Gestational Weight Gain	UoW	University of Warwick
HAPO	Hyperglycemia and Adverse Pregnancy Outcome	US	United States
HC	Head Circumference	WC	Waist Circumference
HMWA	High Molecular Weight Adiponectin	WHO	World Health Organization

Chapter 1

Childhood obesity: the global epidemic

1.1 Introduction

Childhood obesity has reached epidemic levels and constitutes one of the greatest Public Health challenges of the 21st century. It is a condition of excessive fat accumulation in adipose tissue to an extent that general health may be affected (4). Globally, the number of infants and young children (up to 5 years) who were overweight/obese was 42 million in 2013 with numbers expected to increase to 70 million by 2025 (5). United Kingdom's (UK) obesity rate is one of the worst in Western Europe. Health survey for England in 2015 revealed that 28% of children, between 2 and 15 years of age, were either overweight (14%) or obese (14%) (6). Latest data reveal that 1 in 5 children are obese when starting primary school with figures rising to 1 in 3 at secondary school. Most concerning is the fact that in 50% of cases neither children nor their parents are aware about the excessive weight, considering themselves as normal weight (7). By 2020, half children are expected to be obese or overweight. National Health Service (NHS) is currently spending £5.1billion/year on obesity related conditions, constituting the second biggest burden of the economy after smoking. If figures continue to rise, obesity related costs are expected to reach £10billion/year by 2050 (8). Treating obesity has proven to be extremely challenging, therefore is crucial to establish prevention programs at early stages of life. Obesity has previously been considered a high income country problem but increasing rates are now noted in developing countries (9). The transition from rural to urban lifestyle has increased the obesity rates, shifting health problems from malnutrition and infective diseases to cardiovascular events and diabetes. The World Health Organization (WHO) states that social and economic inequities are leading factors for the obesity epidemic, with higher prevalence in low socioeconomic and ethnic minority groups. Consistent with this data, 40% of children in UK deprived areas were obese, compared to 27% in wealthy regions (10).

Recognizing the threat of childhood obesity, English Government set the "Childhood obesity strategy" in 2018, aiming to halve rates within the next 10 years through programs as Change4Life, Living well for Longer and Healthy Lives-Healthy People (11). Cornerstone of the campaign is the multidisciplinary approach to the problem, requiring actions from the government, schools, parents and children themselves. Aim is to tackle the epidemic starting with maternal health and continuing into ways children are grown. Following the "6 pillars in ending childhood obesity" set by WHO (Figure 1.1), program focuses on actions starting from pre-conception and antenatal period, expanding to changes of the obesogenic environment during early childhood and school.

Department of Health emphasizes the correlation between maternal health and offspring's well being. Normal pre-conception Body Mass Index (BMI), early diagnosis of maternal hyperglycaemia and gestational hypertension, gestational weight gain (GWG) within national recommendations and avoidance of smoking, alcohol and toxins during pregnancy ensure a healthy start for the baby. Following the perinatal period, UK government has set a pioneering plan in reducing obesity risk by creating a healthy and supportive environment for children to grow. The introduction of sugar levy as well as the plan to ban advertisements of products high in fat, sugar and salt before 9 pm aims to reduce excessive caloric intake. Implementation of nutritional standards to all schools and promotion of physical activity are other important components of the campaign. Audiovisual media and weight management clinics will be used to inform and support both parents and children on healthy nutrition and lifestyle. Finally, of great importance is the government's statement on supporting and facilitating research relevant to the origins of obesity and ways to prevent it (8).



Figure 1.1: Six pillars of tackling obesity. Measures targeting diet and lifestyle start from preconception and antenatal life and extend to early childhood interventions

1.2 Body Mass Index (BMI)

BMI, defined as the body mass divided by the square of the height (kg/m^2), is used as the reference method assessing obesity. Children are classified as obese or overweight after comparing their BMI with a reference population, adjusting for age and gender. BMI is a measure of weight relative to height, not adiposity. It may be an appropriate tool in adults

(12) but not in children because of the body changes in growth. It cannot distinguish Fat Mass (FM) to Fat Free Mass (FFM) and increased values during adolescence are more likely to reflect FFM rather than adiposity (13). Freedman et al (14) showed that BMI is a good indicator of fatness in children with obesity but in thinner subjects is strongly associated with FFM. Bogalusa Heart study (15) and the Pediatric Roseta study (14) highlighted the need for body fatness cut points, specific to age, gender and race. So far WHO committee has not set any cut off point for %FM in order to define obesity (16).

Evidence suggests that higher BMI is related to short and long term adverse outcomes (17) but there is no data on a specific BMI value. The above has led to a number of reference thresholds causing confusion and inconsistency when reporting obesity rates. There are 4 main classification systems for childhood obesity based on age and gender specific BMI. These are:

1. **Centers for Disease Control and Prevention (CDC)** growth charts, based on data from 4 US surveys. (18) Percentile curves were created after studying North American population and ensured a smooth transition from infants (birth-36 months) to older children (2-20 years) charts. Growth patterns were set after considering ethnic diversity and combined (breast and formula) feeding patterns. Overweight was considered 85th-94th centile and obese >95th centile.
2. **International Obesity Taskforce (IOTF)**, based on data from 6 different countries (19). Data from 97,876 males and 94,851 females from birth to 25 years of age was retrieved in order to provide a universal “cut off” point for obesity. Percentile curves for BMI, sex specific, passing through the adult accepted “cut off” of 25 kg/m² and 30 kg/m² at 18 years of age were created. Children between 2-20 years of age were categorized as normal weight/overweight/obese without information on BMI growth reference.
3. **World Health Organization (WHO)** growth standards (20). In 2006 WHO published growth standards for children aged 0-5 years. Data collected between 1997 and 2003 from 8500 children of different ethnic background was used to create internationally accepted growth curves. Overweight was defined as BMI-for-age Z score ≥ 1 and obesity as BMI-for-age Z score ≥ 2 . In 2007 WHO complimented the previous guidelines by publishing charts for children between 5-19 years, utilizing data from 30,018 children, collected from 1963 to 1974.
4. **UK1990 thresholds.** Nationally representative data from 32,222 children between 1978 and 1994 was used to create centile curves for British population. In clinical settings 91st and 98th centile were used to define overweight and obesity respectively whereas the 85th and 95th centile were used for population surveillance (21).

In order to address the confusion and set clear diagnostic criteria in the UK, the Scientific Advisory Committee on Nutrition (SACN) and the Royal College of Paediatrics and Child Health (RCPCH) published a consensus report in 2009 (22). For children aged 4-18 years, committee suggested the UK1990 criteria to define overweight and obesity, whereas for infants aged 0-4 years the committee recognized the new UK-WHO growth curves. The new charts were based on healthy children around the world who were exclusively breastfed for at least 4 months and have lines drawn at 91st and 98th centile to help clinicians during their assessment.

1.3 Childhood obesity adverse outcomes

The rapidly increasing numbers of children with obesity are followed by short and long term consequences. Metabolic syndrome in adults is known to be related with higher mortality, cardiovascular disease and risk of developing type 2 diabetes (23). On the other hand, metabolic syndrome during childhood is not well described, as the small numbers of children presenting with adverse metabolic profile in the past, didn't allow for safe conclusions. The obesity epidemic had led the International Diabetes Federation (IDF) to develop diagnostic criteria in order to define metabolic syndrome in childhood and adolescence (24). Central obesity is mandatory to establish the diagnosis and is also strongly related to the other components of the syndrome which are hypertension, dyslipidaemia and glucose intolerance (Table 1.1).

Age group (years)	Obesity* (WC)	Triglycerides	HDL-C	Blood pressure	Glucose (mmol/l) or known T2DM
6-<10	≥90 th percentile	Metabolic syndrome cannot be diagnosed, but further measurements should be made if there is a family history of metabolic syndrome, T2DM, dyslipidemia, cardiovascular disease, hypertension, and/or obesity.			
10-<16 Metabolic syndrome	≥90 th percentile or adult cut-off if lower	≥1.7 mmol/l (≥150 mg/dl)	<1.03 mmol/l (<40 mg/dl)	Systolic ≥130/ diastolic ≥85 mmHg	≥5.6 mmol/l (100 mg/dl) (If ≥5.6 mmol/l [or known T2DM] recommend an OGTT)
16+ Metabolic syndrome	Use existing IDF criteria for adults, i.e., Central obesity (defined as waist circumference ≥ 94 cm for Europid men and ≥ 80 cm for Europid women, with ethnicity specific values for other groups*) plus any two of the following four factors: <ul style="list-style-type: none"> • Raised triglycerides: ≥ 1.7 mmol/l • Reduced HDL-cholesterol: <1.03 mmol/l (<40 mg/dl) in males and <1.29 mmol/l (<50 mg/dl) in females, or specific treatment for these lipid abnormalities • Raised blood pressure: systolic BP ≥130 or diastolic BP ≥85 mmHg, or treatment of previously diagnosed hypertension • Impaired fasting glycemia (IFG): fasting plasma glucose (FPG) ≥5.6 mmol/l (≥100 mg/dl), or previously diagnosed type 2 diabetes 				

WC: Waist circumference, HDL-C: High-density lipoprotein cholesterol, T2DM: Type 2 diabetes mellitus, OGTT: Oral glucose tolerance test, *The IDF consensus group recognizes that there are ethnic, gender, and age differences but research is still needed on outcomes to establish risk

Table 1.1: IDF definition of metabolic syndrome in children and adolescents; diagnosing the metabolic syndrome requires the presence of central obesity plus any two of the other four factors. Adopted by Zimmet et al (24)

A cross sectional analysis of 8579 children between 3 and 18 years of age, using data from the National Health and Nutrition Examination Survey (NHANES), revealed that children with obesity have at least two more components of the metabolic syndrome. It also showed that increasing levels of BMI are positively related to hypertension, triglycerides, HbA1c but are negative predictors of HDL cholesterol (25). Sorof et al (26) after studying 2460 students of mixed ethnic background showed that hypertension is more common in children with obesity compared to those of normal weight (33% vs 11%, $p < 0.001$). In agreement with the previous results, Maggio et al (27) using 24hr ambulatory blood pressure monitoring at home and dual energy x-ray absorptiometry, confirmed that increasing levels of fat mass are related to higher systolic and diastolic blood pressure during childhood. Oxidative stress and low grade inflammation are predictors of coronary artery disease and type 2 diabetes (28). High sensitivity C Reactive Protein (hs-CRP), a marker of inflammation, was found to be raised in 22% of overweight and 25% of obese children (29). A study taking place in Turkey reviewed 72 sex and age matched children who were either overweight or had normal weight. The results showed that hs-CRP is higher in children with overweight/obesity and BMI is positively correlated to total and LDL cholesterol but is inversely related to HDL cholesterol (30).

Obesity is a well documented risk factor for type 2 diabetes in adults, but association with paediatric disease is not well studied. The increasing prevalence of type 2 diabetes in childhood, especially in the developing countries of Middle East and Asia, could be attributed to the obesity outburst (31). Al Mamun et al (32) following a cohort of 2,639 Australian participants studied the association of BMI at 5 years of age and the risk of developing diabetes by the age of 21. Results confirmed the role of obesity to the rising incidence of diabetes, as children who were overweight at 5 years had an odds ratio of 2.6 (95%CI: 1.29, 5.22) of experiencing future disease. In 2017, Abbasi et al (33) retrospectively reviewed data from 375 GP practices in the UK. In one of the largest cohorts in paediatric population, data from 369,362 participants, aged 2 to 15 years, was collected from 1994 to 2013, looking for the incidence of type 2 diabetes by the age of 25. Results revealed that childhood obesity was related to four times higher risk for type 2 diabetes when compared to lean subjects. The above observations confirm the role of childhood obesity as a modifiable factor for early onset type 2 diabetes. The obesity epidemic is driving the high prevalence of type 2 diabetes in children and young adults. The early onset of micro and macro vascular complications, characterizing diabetes, will have detrimental effects on future health of this generation.

Childhood obesity was once believed to have only long term cardiovascular effects. Non congenital cardiovascular disease is becoming more frequent, following obesity patterns (34). Altered cardiovascular structure and function are observed in individuals with obesity from the early stages of life. The increased metabolic demand by excess adipose tissue and hypertension lead to cardiac remodeling and left ventricular hypertrophy (LVH) as early as 2 years of age (35). Higher BMI is an independent factor of LVH resulting in impaired systolic and diastolic function (36). Up to date, there is no evidence on the progress of the disease and long term adverse outcomes. Furthermore, children with obesity are found to have increased arterial stiffness and endothelial dysfunction, both prognostic factors of atherosclerosis. In fact, atherosclerotic changes and fatty deposits are found in cadaveric samples starting from 2 years of age (37). The above data suggest that the duration of obesity can be a major determinant of cardiac function and development of heart failure.

Obesity in children and adolescents has adverse effect on multiple organs and tissues of human body. Nonalcoholic fatty liver disease (NAFLD), the most common liver problem in children, can range from steatosis to steatohepatitis and fibrosis. Early development of hepatocellular cancer is related to NAFLD (38). BMI is an independent factor for the disease and is also linked to early formation of gallstones (39). Asthma prevalence is on the rise, currently being one of the leading chronic illnesses during childhood. A UK study of children between 4 and 11 years of age showed that BMI is associated with asthma independent of gender and ethnicity (40). Data from 4000 children in US confirmed that obesity at 2 years is a risk factor for asthma (41). The main pathogenic mechanisms of asthma, bronchial hypersensitivity and airway obstruction, are closely related to obesity. Inflammatory cytokines produced by adipose tissue (IL-6, PAI-1) are responsible for an altered immune response promoting allergic airway disease. The restrictive pattern of obesity causes reduced chest expansion and tidal volumes contributing further to airway hyper responsiveness (42). Similarly to asthma, up to 60% of children with obesity suffer with obstructive sleep apnoea (OSA). Severity of obesity parallels the severity of OSA (43) and each unit of BMI increases the risk of OSA by 12% (44). The altered neuromuscular tone and the mechanical load on the chest are possible explanations for this association. Children with OSA suffer from daytime resulting in reduced quality of life and physical activity, thus creating a vicious circle (45). Furthermore, OSA is linked to systemic hypertension, pulmonary hypertension, ventricular remodeling and arterial stiffness, all recognized risk factors for cardiovascular disease as described before (46, 47).

Musculoskeletal problems are common between children with overweight and obesity. Chronic pain, especially in weight bearing joints, secondary to increased mechanical load results in reduced physical activity and further weight gain (48). Excessive adipose tissue affects bone development leading to fractures. Children with obesity have an increased odd ratio of 1.23 (95% CI: 1.12, 1.35) of acquiring a low impact fracture when compared to normal weight (49). Mechanical reasons (increased forces due to weight) and the effect of cytokines produced by adipose tissue in promoting osteoclastic activity are possible explanations for the above observation. Childhood obesity is characterized by insulin resistance and an imbalance between androgens and oestrogens (50). Polycystic ovarian syndrome (PCOS), a common endocrinology problem in adolescents, presents with amenorrhoea, hirsutism and acne due to excessive androgens in female patients. Except from the established cardiovascular risk, girls with PCOS have a negative perception of their body image leading to low self esteem, depression and even suicidal attempts (51).

Childhood obesity is related not only to medical but also to psychosocial aspects. Children are victimized (bullied) due to excessive weight as early as 3 years of age (52). Beliefs such as “obese individuals lack willpower and self-discipline” and “obesity is the characteristic of a weak person” are common in “obesity stigma”. Lumeng et al (53) showed that children with obesity are more than twice likely to be bullied compared to normal weight and weight discrimination rates in adolescence reach those of sexual orientation. Weight based victimization leads to low self esteem, depression and impaired social and academic performance (54). Youth with obesity are socially isolated because of their body habitus and find it difficult to get into a relationship. School is the main environment of victimization, leading children to poor attendance and performance. Bullied youth focus on ways to avoid public harassment instead of concentrating on academic requirements. Finally, discrimination during physical activities, at school or at the gym, leads to adverse behaviors such as binge/emotional eating and sedentary lifestyle, enhancing further the obesity loop (55).

The correlation between obesity and medical co-morbidities is well described. Adults with obesity are at higher risk of type 2 diabetes mellitus, coronary artery disease and strokes, asthma, osteoarthritis, gallstones and all cancers except oesophageal and prostate (56). Children with obesity will develop adult obesity, therefore are at higher risk of adverse medical outcomes. Simmonds et al (57) in a meta-analysis of 200,777 participants, using BMI “cut off” to define obesity, showed that children with obesity are five times more likely to develop adult obesity when compared to normal weight. Results revealed that 70% of cases of obesity during adolescence will remain obese after the age of 30 years.

These findings are in agreement with previous work performed by Parsons et al (58) in 1999. Risk prediction models estimate that in the next 20 years 100,000 new coronary events would be attributed to childhood obesity (59). In cadaveric models, obesity was shown to predict the extent of coronary artery atherosclerosis in adolescents and young adults (60). Adolescent BMI, was an independent predictor of coronary artery disease in a 17 years follow up study of Israeli population (61). Furthermore, in the Cardiovascular Risk in Young Finns study, cardiovascular disease risk factors in childhood (BMI, hypertension, dyslipidaemia) correlated with values in adulthood (62). Franks et al (63) showed that childhood obesity, glucose intolerance and hypertension increase the rates of premature death in an American Indian population. In summary, childhood obesity and the related medical, socio-economic consequences seem to persist into adulthood. Tackling the rising rates of obesity in the early stages of life may therefore reduce the obesity epidemic seen in adults.

1.4 Causes of childhood obesity

1.4.1 Diet and Lifestyle

Obesity is difficult to treat, therefore investigating the origins of the disease and forming prevention strategies is critical. The phenomenal increase in childhood obesity is multifactorial (Figure 1.2). It is primarily caused by an imbalance between energy intake and expenditure. The positive energy balance is associated with the modern lifestyle adopted by children. Davidson et al (64) described the 'ecological model' where obesity is related to dietary intake, physical activity and sedentary behaviour.

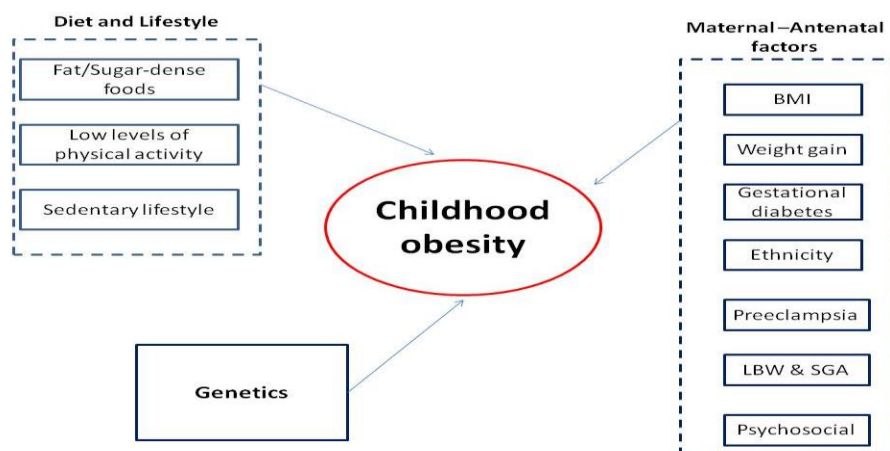


Figure 1.2: Causes of childhood obesity. The pathogenesis includes genetics, antenatal and environmental factors; LBW: low birth weight, SGA: small for gestational age

Children's dietary patterns are central in the development of overweight. Poor diet containing high levels of fat or sugar is contributing to 'unhealthy' weight gain. Fast food, candy, soft drinks and convenience meals, such as canned pastas and frozen dinners are diet choices progressively leading to excessive fat levels in the body (65). Children with obesity consume 500 extra calories per day and 3 times higher sugar than recommended. Sugary drinks account for the 30% of daily sugar intake (66). The rapidly changing diet patterns are a result of "Westernization" and "Dietary Liberalization", described by Gupta et al (67). Children have unrestricted access to fast foods and sugary drinks in shops and vending machines located close to their school. The daily "money in the pocket" allows them to purchase their lunch which in combination with the lack of knowledge on nutritional components lead to unhealthy dietary options. Children are an easy target group for big food companies. Aggressive advertisement and low cost products lead to increased consumption of energy dense foods. UK Government, recognizing the above hazard and in an attempt to prevent unhealthy food options, introduced school food standards and legislation to restrict food marketing to children.

Traditional counterfactual beliefs on health and nutrition, passed from generation to generation, have always contributed to childhood obesity. Misconceptions such as "fat baby is a healthy baby", "girl's weight reflects her wealthiness" and "children's fat goes away as they get older" have led to unhealthy diet behaviors (67). Forced feeding at any age group and excessive feeding of babies born small for their age are common practices seen in many ethnic groups. Parents and grandparents have limited knowledge on nutrition and even homemade food can be harmful for child's health when wrong ingredients are used. For example, a study in Greece revealed that children living at homes where the grandmother was cooking were more prominent to obesity (68).

High levels of physical activity could potentially compensate for the increased caloric intake, allowing the maintenance of a normal weight. The health benefits of physical activity are well described (69). The US Physical activity guidelines are in concordance with the UK guidance (8) recommending that children and adolescents aged 6 to 17 years should have 60 minutes (1 hour) or more of physical activity each day. Unfortunately only 21.6% of 6 to 19 year old children and adolescents in the US and 28% in UK achieved this target in 2016 (70). Similar recommendations exist for children below 5 years of age. Babies should be encouraged to pull, push, reach, grasp and spend tummy time on daily basis. Toddlers able to walk should be physically active (climbing, riding, chasing games, rolling and skipping) for at least 3 hours per day. Child characteristics, such as gender, age, body weight, may affect the physical activity levels. Reduced physical activity rates with increasing age may be related to puberty and its

associated physical, emotional, and social changes (64). Females are known to be less active than males. Sociocultural beliefs against outdoor playing and the engagement with household jobs are main factors for the increased obesity rate seen in females. Furthermore, children are under constant pressure to perform academically, hoping for a better future and working potentials, neglecting at the same time sports (67). Lack of safe infrastructure for walking, running or cycling to school has led 53% of UK primary school children to commute by bus or car in 2012, compared to 80% who walked independently to school in 1971 (71).

Sedentary lifestyle, with children spending many hours on indoor activities, such as video games, social media and watching television, is another important factor for obesity. In 1995, a child spent on average 3 hours/day in front of a screen compared to 6 hours today. Lack of playgrounds and unsafe neighborhoods, characteristics of our modern society, may be driving the above observation (66). Snacking and craving for food is closely related to sedentary lifestyle (72). Story et al (73) showed that number of hours spent in front of TV correlate with consumption of most advertised goods, including foods high in fat, sugar and salt. The unrealistic body ideals promoted by social media, lead to frustration reactivity, self neglect and low self esteem resulting into unhealthy dietary patterns.

1.4.2 Genetics

Children with obesity tend to have parents who also suffer from excessive weight (74). Genetics is another factor contributing to obesity. 25-40% of BMI is heritable whereas 50% of the way fat is distributed is attributed to heritability (75-77). Identical twins raised apart have a 0.7 correlation in BMI, similar to those raised together (0.74) (78). More than 75 genes and loci have been identified so far contributing to obesity. Leptin gene (Ob) deficiency, FTO and MC4R genes and mutations contributing to low basic metabolic rate (BMR) and low rate of lipid oxidation are some of the most well described (79, 80). More likely gene effects are responsible for the susceptibility of developing obesity in the presence of an adverse environment rather than the obesity expression itself. In this regard, Heitmann et al (81) showed that high fat diet resulted in weight gain only in those subjects who were already overweight or had parents with obesity. Furthermore, trying high fat diet in paired of twins resulted in higher correlation weight gain within twin pairs compared to “between” twin pairs, confirming the role of genetics in proneness of developing obesity (82). As the gene pool remains unchanged, genetic susceptibility accounts for <5% of childhood obesity, therefore it cannot entirely explain the increase in

obesity rates. Genetic factors need to be coupled with environmental in order to affect weight (83), therefore the main focus of research against the rising trends of obesity should be on energy balance and events occurring during early stages of life.

1.4.3 Intrauterine programming

For humans, in utero life is a plastic period sensitive to environmental factors resulting in the creation of phenotypes better matching their environment. Plasticity allows fetuses to get prepared for the world they are going to live in. During fetal life body goes through critical periods of development. “Programming” is an event that has lasting or life-long effects (84). Barker et al (85) was the first to describe the “fetal origins hypothesis”, suggesting that diseases in adult life originate from adverse intrauterine environment during developmental plasticity. Since then, many longitudinal studies have shown that despite genes and lifestyle may contribute to the development of obesity, it is the fetal period which remains critical for the metabolic risk (86, 87). Maternal BMI and GWG, ethnicity, birthweight, gestational diabetes mellitus (GDM) and postnatal feeding mode are factors contributing to the developmental programming of obesity and metabolic disease (Figure 1.3) (88).

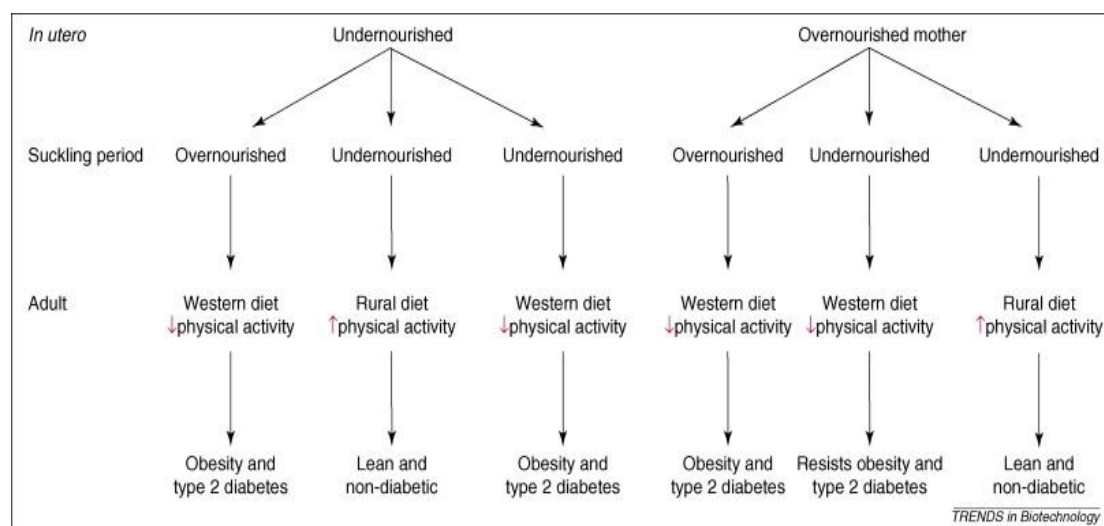


Figure 1.3: Impact of intervening in early post natal life. Favorable effects present when antenatal and postnatal environment are in concordance; Adopted from Stocker et al (89)

The “programming factor” and the exact mechanism underpinning intrauterine programming are yet to be identified (90). Barker et al (84) based on the “life history theory” suggested that during pregnancy the cost of allocating energy to vital organs at

the expense of other traits results in disease in later life. Specifically, in conditions of maternal undernutrition, energy is allocated to the development of brain resulting in fewer cells in key organs. People who were small at birth have lower numbers of renal glomeruli and reduced pancreatic beta cell mass leading to early glomerulosclerosis, hypertension (91) and diabetes (92) respectively. Hormones and metabolism may also explain the link between adverse fetal environment and future disease. Fetuses with reduced maternal supply develop peripheral insulin resistance to allow adequate glucose levels for brain development, sacrificing muscle growth and sensitivity to insulin action, leading to impaired glucose tolerance and type 2 diabetes in the future (93, 94).

The role of hypothalamus in the adverse metabolic programming has drawn the attention of many researchers. Hypothalamus and particularly the arcuate nucleus (ARC) is the primary site for energy regulation. Development and maturation of hypothalamus and its projections occurs during fetal and immediate postnatal life in humans (95). Insulin and leptin receptors have been identified in the ARC neurons (96). The involvement of hormones in the development of hypothalamus makes it vulnerable to maternal metabolic state. Intrauterine obesogenic environment leads to increased fetal/neonatal insulin and leptin levels altering hypothalamic development. Neurogenesis of neurons with orexigenic effect is promoted and projections from the ARC to paraventricular nuclei are reduced resulting in future hyperphagia and eventually obesity (97, 98).

In terms of timing, gestation and the early post natal period are critical for “metabolic programming”. Adverse events during specific periods of gestation have various results on the offspring. In animal models, maternal undernutrition in early pregnancy is linked to increased adiposity after birth whereas nutrient restriction during the latter part results in lean phenotype (99, 100). Results are consistent with the epidemiological data from the Dutch Hunger Winter findings where fetuses exposed to famine during first trimester had higher obesity risk as adults whereas those exposed during third trimester had lower rates (101). Postnatal exposure to adverse environment is equally important to in utero. Obesity-prone pups fostered by lean dams in the immediate postnatal period show improved metabolic profile and on the other hand lean pups fostered by obese dams develop increased adiposity (102). Vogt et al (98) revealed that exposure to maternal obesity only in the postnatal period is enough to create adverse metabolic profile. Possible explanation is that the high levels of free fatty acids, glucose, insulin and leptin present in the milk of mothers with obesity during lactation alter hypothalamic structure, leading to hyperphagia and obesity.

Prevention of obesity starting from prenatal life can have multigenerational impact. Children with obesity will remain obese in adolescence and adulthood. In cases of female adolescents, pregnancies complicated by obesity will result in offspring at high risk of developing future obesity, thus creating a vicious transgenerational cycle. It is therefore of great interest to investigate the potential epigenetic mechanisms that determine these risks, which will then allow to develop intervention strategies during the peri-conceptual and immediate postnatal period that can modify/reduce them.

1.4.4 Role of maternal factors on the pathogenesis of childhood obesity

1.4.4.1 Gestational Diabetes (GDM)

GDM is glucose intolerance of different degrees that is first recognized during pregnancy. The increasing incidence of GDM, which can complicate 5-25% of pregnancies depending on the diagnostic criteria used and population studied, is in parallel with the obesity outburst. It is the commonest medical complication in pregnancy, related with short and long term complications and increased morbidity for both mother and the baby. Worldwide, 17.7 million pregnancies were complicated with GDM in 2015, with 1 in 7 births affected in 2017 (103). South East Asian region has the highest prevalence, followed by Middle East and North Africa. Reduced access to maternal care, noted in low and middle income countries, is associated with higher rates of GDM. The growing burden of GDM is driven by the obesity outburst and by the fact that women tend to be more overweight or obese than men (104).

GDM seems to be a result of the same physiological and genetic abnormalities that define diabetes outside pregnancy (105). There are two main key points that characterize normal pregnancy and play a major role in the pathophysiology of GDM. First is the progressively worsening insulin resistance during pregnancy that reaches a peak during the third trimester. Secondly is the increased insulin secretion by pancreatic β -cells in order to balance this high insulin resistance. The main abnormality in GDM is that insulin supply is not adequate to counterbalance insulin resistance and regulate the degree of hyperglycaemia. B-cell dysfunction, occurring on a background of pre-existing chronic insulin resistance (obesity, Asian origin, polycystic ovarian syndrome) or caused by autoimmune or monogenic mechanisms, is the major contributing factor (106). Less than 10% of women with GDM have autoantibodies against islet cell or Glutamic Acid Decarboxylase (GAD) and these women are usually lean and can rapidly develop diabetes postpartum. Monogenic type of GDM also accounts for less than 10% of cases

(107). Hormones such as estrogen, progesterone, cortisol and placental lactogen, progressively increase during pregnancy, peaking at the third trimester, causing higher insulin resistance. Adipocytokines (TNF α , adiponectin, leptin, resistin) and lipid concentrations have been shown to be related to changes in insulin sensitivity both in pregnant and non-pregnant women. Normal pregnancy is characterized by reduced adiponectin levels and GDM pregnancies have been found to have even less, attributing to higher insulin resistance. On the other hand, raised levels of TNF α , resistin and leptin have been correlated negatively with insulin sensitivity during pregnancy (108).

1.4.4.1.1 Diagnosis of gestational diabetes

There is no consensus on the optimal screening and diagnostic criteria of GDM. Despite extensive research, guidelines vary globally and are adjusted according to infrastructure, cost effectiveness and patients' needs (109). When and how to screen for GDM, universal versus selective screening are still questions to be answered (Table 1.2).

In 1964, O'Sullivan and Mahan formed for the first time criteria to diagnose GDM. They suggested a 50gr 1hr Glucose Challenge Test (GCT) to screen for GDM followed by a confirmatory test (in those who were GCT positive) with a 100gr 3hr Oral Glucose Tolerance Test (OGTT). In the following years the Somogyi-Nelson method, used by O'Sullivan and Mahan to measure blood glucose, was replaced by more specific enzyme assays based on plasma and not whole blood. The above resulted in the formation of new criteria and thresholds for the diagnosis of GDM.

In 2004, American Diabetes Association (ADA) recommended early screening of high risk pregnancies (Table 1.3). A Fasting Plasma Glucose (FPG) $\geq 7\text{mmol/l}$ or a random plasma glucose $\geq 11.1\text{mmol/l}$ would be diagnostic of pregestational diabetes. For women not previously diagnosed with overt diabetes, testing for GDM should be performed at 24-28 weeks of pregnancy following one (75gr OGTT) or two step process (50gr GCT followed by a diagnostic 100gr OGTT).

Table 1.2: Various criteria proposed for diagnosing GDM based on fasting OGTT (24-28 weeks gestation)

Criteria	Year proposed	Approach	Glucose load (g)	Glucose threshold mmol/l (mg/dl)			
				Fasting	1 h	2 h	3 h
O'Sullivan & Mahan	1964	2 step	100	5.0 (90)	9.2 (165)	8.1 (145)	6.9 (125)
National Diabetes Data Group (NDDG)	1979	2 step	100	5.8 (105)	10.6 (190)	9.2 (165)	8.1 (145)
Carpenter & Coustan	1982	2 step	100	5.3 (95)	10.0 (180)	8.6 (155)	7.8 (140)
World Health Organization (WHO)	1999	1 step	75	7.0 (126)	–	7.8 (140)	–
American Diabetes Association (ADA)	2004	2 step	100	5.3 (95)	10.0 (180)	8.6 (155)	7.8 (140)
Latin American Diabetes Association (ALAD) ^b	2008	2 step	75	5.5 (100)	–	7.8 (140)	–
International Association of Diabetes and Pregnancy Study Groups (IADPSG)	2010	1 step	75	5.1 (92)	10.0 (180)	8.5 (153)	–
WHO 2013 criteria (revised, same as IADPSG)	2013	1 step	75	5.1 (92)	10.0 (180)	8.5 (153)	–
National Institute for Health and Care Excellence (NICE)	2015	1 step	75	5.6 (101)	–	7.8 (140)	–

Adopted from Vandorsten et al (110)

Table 1.3: Risk factors to screen for GDM as per ADA guidelines

-
- Age ≥ 25 years
 - BMI ≥ 25 kg/m² and BMI ≥ 23 kg/m² in Asian Americans
 - High risk ethnic groups: South Asian, Aboriginal, Hispanic
 - Previous history of GDM
 - Family history of type 2 diabetes
 - History of poor obstetric outcomes: congenital malformation, still birth etc
-

The need for widely accepted criteria based on maternal and infant adverse outcomes triggered the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study. 23,000 pregnant women had OGTT between 24-32 weeks gestation and a linear relationship between glucose levels and adverse outcomes was demonstrated without any clear cut off point (111). Following the HAPO study results, the International Association of the Diabetes and Pregnancy Study Groups (IADSPG) suggested thresholds for the diagnosis of GDM and overt diabetes at first as well as third trimester (24-28 weeks gestation) (Table 1.4). There has been a lot of debate and controversial evidence regarding the low FPG cut off of 5.1mmol/l (92mg/dl) (112, 113) but general consensus to the 24-28 weeks criteria. World Health Organization (WHO), Endocrine Society, the Australian Diabetes in Pregnancy Society (ADIPS), the International Federation of Gynecology and Obstetrics (FIGO), the European Association for the Study of Diabetes (EASD) and the European Board and College of Obstetrics and Gynecology (EBCOG) have accepted 2hr 75gr OGTT at 24-28 weeks gestation as the optimal screening test for GDM.

In 2015, National Institute of Clinical Excellence (NICE) published guidelines for the diagnosis of GDM. While IADSPG criteria are based on reducing maternal and offspring harm, NICE are based on cost effectiveness studies using health economics. It is recommended that women falling into one of the risk groups (Table 1.5) should have a 75gr OGTT at 24-28 weeks of gestation (105).

Table 1.4: IADSPG criteria for GDM diagnosis. Screening includes investigations at first antenatal visit but also at 24-28 weeks gestation

First prenatal visit	Measure FPG, HbA1C or RPG	Over diabetes if, FPG ≥ 7.0 mmol/l (126mg/dl) or RPG ≥ 11.1 mmol/l (200mg/dl) or HbA1c $\geq 6.5\%$ (48mmol/mol) GDM if FPG ≥ 5.1 mmol/l (92mg/dl) but <7.0mmol/l (126mg/dl)
If the test is normal in the first prenatal visit, test for GDM during 24-28 weeks		
24-28 weeks gestation	75gr OGTT	Pre-existing diabetes if FPG ≥ 7.0 mmol/l (126mg/dl) GDM if FPG ≥ 5.1 mmol/l (92mg/dl) 1hr ≥ 10.0 mmol/l (180mg/dl) 2hr ≥ 8.5 mmol/l (153mg/dl)

Table 1.5: Risk factors to screen for GDM as per NICE guidelines. NICE suggests selective screening based on cost-effectiveness data

-
- BMI above 30kg/m²
 - Previous macrosomic baby weighing 4.5kg or above
 - Previous gestational diabetes
 - Family history of diabetes (first degree relative)
 - Minority ethnic family origin with a prevalence of diabetes
-

In an ideal world, author believes that universal criteria should be used for screening and diagnosing GDM. Health resources, economics and cultural differences don't allow a consensus view. Further research and studies will be required to address the existing problem.

1.4.4.1.2 Gestational Diabetes consequences

In 1920, Jorgen Pedersen formulated the hypothesis which states that "Maternal hyperglycaemia results in fetal hyperglycaemia and hence in hypertrophy of fetal islet cells with insulin hypersecretion, leading to greater fetal utilization of insulin" (114). The intrauterine hyperglycaemic state is toxic for mother and fetus, resulting in short and long term adverse outcomes (Figure 1.4).

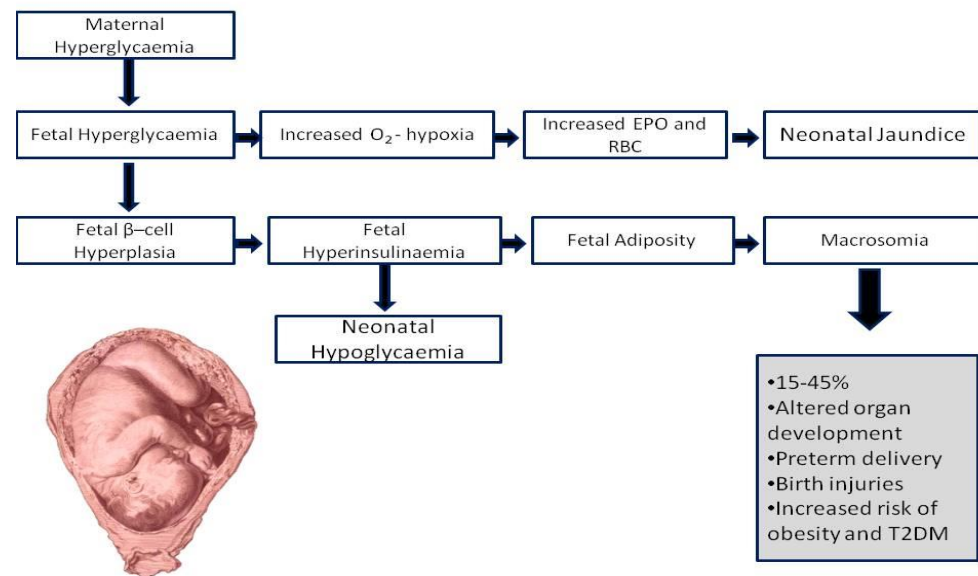


Figure 1.4: Pedersen hypothesis. Common denominator for the complex pathophysiology of GDM is maternal hyperglycaemia; EPO: erythropoietin, RBC: red blood cells, T2DM: type 2 diabetes mellitus

1.4.4.1.3 Short term effects on the offspring

The association between pregestational diabetes and congenital malformation is well established. Balsells et al (115) has shown that even women with gestational diabetes have a higher risk for cardiac (ventricular septal defect), neural (caudal dysplasia), gastrointestinal and genitourinary malformation compared with general population [Relative Risk (RR): 1.16, 95% CI 1.07-1.25].

Chronic maternal hyperglycaemia leads to glycosylation of haemoglobin, reducing the capacity to carry oxygen. The hypoxic environment combined with the increased fetal oxygen demand due to hyperinsulinaemia leads to increased erythropoiesis and polycythaemia. Red blood cell breakdown will cause neonatal hyperbilirubinaemia. If the fetal adoptive mechanisms are not enough to counterbalance the hypoxaemia, anaerobic

metabolism promotes lactate production and acidaemia, resulting in intrauterine death. Fetal exposure to high glucose levels cause hyperplasia of fetal pancreatic β -cells and hyperinsulinaemia. Raised insulin levels inhibit accumulation of Surfactant Protein A and B (SP-A and SP-B) messenger RNA (mRNA) and antagonize cortisol effect on the development of lung morphology and surfactant system in type II cells. The above effect, results in increased rates of neonatal Respiratory Distress Syndrome (RDS) (116).

GDM diagnosis and management leads to reduced threshold for caesarian section (CS) as preferred method of delivery, independent of birth weight (117). GDM by itself is not an indication for CS though and mode of delivery should be a shared decision between clinician and mother. Specific conditions such as macrosomia, fetal distress and risk of intrauterine death are factors leading to abdominal delivery (118). Prematurity of the babies and the excessive retention of fluid in the lungs associated with CS are the main reasons for "iatrogenic RDS" (119). Furthermore, the association of GDM with increased risk of stillbirth and excessive fetal growth during the latest stages of pregnancy has led clinical practice to earlier induction of labour. Benefits of this approach always need to be weighed against the possibility of higher rate of CS, maternal infection, neonatal mortality and morbidity (120).

Macrosomia, defined as birth weight $>4\text{kg}$, is the most well described complication of GDM. Hyperglycaemia in the fetus stimulates insulin and growth hormone production, promoting fat and glycogen deposition through their anabolic effect. Macrosomia presents in 50% of GDM pregnancies and the risk is 10 times higher than general population. Shoulder dystocia (SD), the impaction of neonate's shoulder against maternal pelvis, is strongly related with macrosomia (121). SD and obstructed labour can lead to perinatal complications, like Erb's palsy (form of brachial plexus palsy) and birth asphyxia. HAPO study revealed a direct association between increasing maternal glucose and increased risk of large for gestational age (LGA) babies, macrosomia and SD (111).

GDM is not only related to adverse neonatal size but also to altered body composition. Whitelaw in 1999 (122) was the first to demonstrate an association between maternal glycaemia and offspring's skinfold thickness. Sparks et al (123) described that fat mass at birth is related to intrauterine environment whereas lean mass is more closely linked to genetic factors. Anthropometric measurements on 19,389 neonates from the HAPO cohort revealed a strong association between maternal glycaemia and neonatal adiposity. In particular, maternal glucose levels were stronger associated with subscapular skinfold thickness (marker of central adiposity) and the association was

mediated by fetal insulin, implying that adverse phenotype is present from intrauterine life (124). To confirm the hypothesis, Venkataraman et al (125) assessed sonographic data from GDM pregnancies in India at 11, 20 and 32 weeks gestation. Results showed that GDM fetuses had increased anterior abdominal wall thickness even at 20 weeks of gestation (even before GDM was diagnosed), with findings persisting to 32 weeks, irrespective of GDM treatment. Throughout the measurements, GDM babies were consistently smaller in size, having the typical “thin but fat” phenotype. The above findings suggest that being exposed to obesogenic and hyperinsulinaemic environment, fetuses show a preferential growth for the insulin sensitive adipose over fat free tissue. Catalano et al (126) and Lingwood et al (127) using direct measures of infant adiposity confirmed that adverse body composition is present at birth in the GDM group. Many researchers argued that increased adiposity at birth may be related to maternal BMI rather than the hyperglycaemia effect. Logan et al (128) performed a systematic review which identified 27 studies showing that GDM neonates have increased fat mass, percentage of body fat and similar fat free mass compared to controls, with results remaining unchanged after adjustment for maternal BMI. However, poor quality of studies, using skinfold thickness rather than direct adiposity measures and not adjusting for maternal HbA1c were some of the main limitations of the reviewed literature. Catalano et al (129) studying a subgroup of the HAPO cohort who developed GDM found that maternal hyperglycaemia has a higher impact on birthweight and adiposity at birth compared to maternal BMI, with their combination having the strongest effect. Overall, studies suggest that the hyperinsulinaemic intrauterine environment in GDM pregnancies leads to adverse body composition at birth with increased neonatal fat mass. Tracing this altered phenotype in childhood and adolescence will confirm the developmental origins of obesity and will allow intervention strategies targeting early life body composition.

Intrauterine Growth Restriction (IUGR) is noted in <5% of GDM pregnancies (130). The exact pathogenesis remains unclear and different mechanisms have been described. Placental vascular insufficiency resulting in fetal malnutrition and hypoxia, as well as the higher rate of maternal hypertension noted in GDM mothers are the most prevalent explanations.

Uncontrolled maternal hyperglycaemia is associated with major complications in the immediate postpartum period. It is well described though, that treatment of GDM reduces the risk of adverse outcomes. The ACHOIS trial in 2005, showed that intensive GDM treatment reduces the risk of perinatal complications (RR 0.33, 95% CI 0.14-0.75), macrosomia (RR 0.47, 95% CI 0.34-0.64), LGA and birth weight compared to routine care (121). Similar results were demonstrated in two large systematic reviews: In 2010,

Horvath et al (131) demonstrated reduced risk of SD, LGA and macrosomia with intensive GDM treatment and Poolsup et al in 2014 (132) added that therapeutic interventions also reduce risk for gestational hypertension (RR 0.68, 95% CI 0.53-0.87) compared to standard care.

1.4.4.1.4 Long term effects on the offspring

The chronic exposure of the fetus to a hyperglycaemic environment increases the risk of an adverse metabolic profile in future life. There is accumulating evidence of the developmental origins of obesity, with incidents occurring during intrauterine or early post natal life having long lasting effects on the offspring (133). In a UK cohort of 42 GDM and 44 controls adipose tissue was measured by MRI at birth and 3 months later. Despite similar adipose tissue volumes at birth, GDM offsprings were found to have 16% higher volumes at follow up visit, adjusting for maternal BMI, gender, ethnicity and mode of feeding and irrespective of good glycaemic control. GDM group had greater weight gain, driven by adipose tissue accumulation (134). The mechanisms underpinning the above observations remain unclear and many potential mechanisms have been suggested. The obesogenic environment in utero leads to higher fetal and neonatal free fatty acid, glucose and insulin levels. The increased levels of these biomarkers result in altered hypothalamic programming causing impaired satiety and hyperphagia (90). Preliminary data suggests that breast milk from mothers with diabetes has altered composition with more glucose and insulin, thus leading to adipose phenotype (135). Plagemann et al (136) after comparing mothers with diabetes feeding their infants with their own milk against those using non diabetic banked donor breast milk found that the earlier group had higher risk of overweight and impaired glucose tolerance at 2 years of age. Finally, factors other than hyperglycaemia which are closely related to pregnancies complicated by diabetes may contribute to the obesity origins. Maternal hypertriglyceridaemia and low HDL levels are linked to increased fetal growth (137, 138) and are consistent with the “fuel mediated teratogenesis” described by Freinkel (139).

Gestational diabetes is linked to future obesity and metabolic dysfunction (140). Exposure to diabetes in pregnancy is related to increased BMI, total and abdominal adiposity in childhood and adolescence (141, 142). Visceral fat is one of the strongest predictors in the development of metabolic syndrome (143). Follow up studies from the HAPO cohort have confirmed that maternal hyperglycaemia is linked to impaired fasting glucose, impaired glucose tolerance, raised HbA1c and reduced insulin sensitivity by the age of 14 years (144). This adverse metabolic profile is also present in adulthood with

adults exposed to GDM having two and four times higher risk for overweight and metabolic syndrome respectively (145). Studies on Pima Indians, confirmed that offsprings of pregnancies complicated by GDM have higher risk of developing adult obesity compared to the offsprings of mothers who developed diabetes later in their lives or those who never developed diabetes (146).

During the last decade, researchers have been arguing whether the long term adverse effects of GDM are mediated by maternal BMI and birthweight in genetically predisposed individuals. Diabetes in pregnancy is more commonly seen in mothers with obesity and raised maternal BMI is linked to future metabolic dysfunction (147). Two systematic reviews addressing the association between GDM and future obesity provided inconsistent results as the majority of the studies did not adjust for maternal BMI (148, 149). Thaware et al (150) following 1,320 participants from the HAPO study concluded that maternal BMI accounts for any association between mild maternal hyperglycaemia and obesity at 7 years of age. Consistent with these findings, the UK ALSPAC cohort (n=6,842) showed that the association between GDM and central/total adiposity at 10 years of age becomes non significant after adjusting for maternal BMI (151). On the other hand, two large studies following a total of 28,263 children at the age of 10 provided strong evidence that GDM is related to increased obesity even after controlling for maternal BMI (152, 153). Trying to explain these contradictory results, it was suggested that the effects of GDM on future metabolic function may manifest later than the studied age groups. Consistent with this hypothesis, the HAPO follow up study at 14 years after delivery confirmed an impaired glucose tolerance irrespective of maternal BMI (144). Another potential explanation for the above findings is that studies failing to show an independent association between maternal glucose levels and future obesity included only cases with mild GDM, suggesting that a higher maternal glycaemic threshold may amplify the association.

Offspring of pregnancies complicated by GDM are at higher risk of being SGA, LGA or macrosomic, as already described. Longitudinal studies in different ethnic groups have described a "U" shaped association, with increased risk of developing type 2 diabetes and obesity at both ends of the distribution (Birth weight <2.5kgr and >4kgr) (154). Hillier et al (155) in a cohort of 9,439 children revealed that GDM is linked to both neonatal macrosomia and childhood obesity at 7 years of age. Applying multiple regression models showed that the GDM effect on future obesity is independent of the size of the offspring at birth. Finally, in order to assess the long term effects of GDM, independent of genetic and lifestyle contributors, two main studies have compared within siblings effect before and after the development of maternal diabetes in pregnancy. In a sibling study of

Indian population (n=182) offspring born after the development of GDM had higher rates of obesity and type 2 diabetes when compared to their siblings born before their mother's diagnosis (156). As Pima Indians is a high risk group and in order to generalize the results to other populations, a large prospective (n=280,866) study of Swedish population was conducted. Consistent with findings in Pima Indians, Swedish siblings born after the GDM diagnosis had a greater BMI of 1.23kg/m² (95%CI: 0.11, 2.36) compared to their siblings born before the diagnosis. Results remained unchanged after adjustment for covariates including maternal BMI and birthweight, implying that the adverse effect of GDM on future metabolic profile is due to intrauterine programming (157). In conclusion, up to date data suggests that fetal exposure to a hyperinsulinaemic environment leads to adverse metabolic programming, with lifestyle characteristics, common environments and maternal genotype being contributing factors.

1.4.4.1.5 Short term effects on the mother

Poor glycaemic control in pregnancies complicated by diabetes increases the risk of maternal urinary tract, wound and endometrial infections (158). Increased incidence of hypertension is also described during GDM. Despite mothers already having co-existing risk factors (obesity, family history), GDM remains an independent risk, increasing the ratio by 1.67 and 1.54 for hypertension and pre-eclampsia respectively (159). Results from the HAPO cohort confirm that increasing levels of maternal glycaemia are linked to raised blood pressure. The exact mechanism is not well described but insulin resistance seems to be one of the main contributing factors. The increased incidence of CS and labour induction has already been discussed.

1.4.4.1.6 Long term effects on the mother

Pregnancy is a period in life where β -cell dysfunction can be unmasked, due to increased insulin resistance, leading to abnormal blood glucose levels. Mothers who develop GDM have seven times higher risk of developing type 2 diabetes in future (160). Incidence of diabetes is 3-70% depending on the diagnostic criteria used and length of follow up (161). Prevalence is higher during the first five years and plateaus after ten. Different studies have suggested various predictors with fasting blood glucose during antenatal OGTT (161), pre-pregnancy BMI, intrapartum and post partum weight gain and insulin treatment (162) having the strongest effect. A recently published meta-analysis of primary care data from UK, including 9,118 women with GDM matched with 37,281 controls, revealed that GDM mothers are 20 times more likely to develop diabetes, 2.7

times more likely to develop coronary disease and twice as likely to develop hypertension in the next 25 years (163). Mothers with GDM are at risk of diabetes recurrence in subsequent pregnancies, with 3 out of 5 pregnancies been complicated by maternal hyperglycaemia. The magnitude of the risk increases with the number of previous GDM episodes (164).

There is global consensus that the risk of future diabetes minimizes with lifestyle changes. Normalization of BMI through healthy diet and graded physical activity is the cornerstone in the prevention of future diabetes. GDM mothers should adhere to postpartum diet with energy deficit of 500-1000 calories/day in order to achieve weight loss of 0.5-1kgr/week. Graded activity of 30 minutes for 5 days/week is also advised. Aerobic exercise such as brisk walking, yoga and swimming combined with strength training with light weights and elastic bands are also recommended. Great emphasis needs to be given to post-partum advice and education. All mothers require counseling emphasizing on ways to prevent future metabolic risks and the need to attend annual screening appointments for diabetes. Breast feeding advice should be provided and the protective role against the development of type 2 diabetes explained (165).

1.4.4.1.7 Management of Gestational Diabetes

Self-care is of vital importance in the management of GDM. As soon as the diagnosis is formed, information and education to the patient in order to facilitate self-care should be provided and the effects of diabetes in pregnancy should be explained. Lifestyle changes are the most important intervention in GDM treatment, as they provide adequate control in the majority of cases. Pregnant women are advised to follow a balanced diet tailored to their needs. Low glycaemic index foods and restriction of carbohydrates to 40% of the daily caloric intake are general recommendations (105). GWG should be based on the early pregnancy weight, as per the Institute of Medicine (IOM) guidelines (166). In the GDM group, target should be set 10% lower from the recommended weight gain in normal pregnancy. Maternal starvation and weight loss are not recommended, as they are linked with fetal malnutrition and IUGR (167). Regular exercise is beneficial in GDM by increasing insulin sensitivity and glucose absorption by the skeletal muscles. Pregnant women are advised to have 150 minutes of mild to moderate exercise per week.

When treatment targets are not achieved with lifestyle changes, a medical approach is required. Injectable insulin and oral antidiabetic agents are the available options. When

administering drugs during pregnancy, safety of both mother and fetus should be considered and benefits should outweigh any potential risks.

Insulin treatment is globally considered the safest option for GDM treatment with an immediate effect on maternal glycaemia. Human insulins do not cross the placenta and are not related to fetal complications (168). Insulin treatment can lead to tight glycaemic control, but caution is required to avoid hypoglycaemic episodes as they are related to growth and developmental deficits. Recurrent episodes in the third trimester may be an indication of placental insufficiency (169).

Currently, there are 2 oral agents licensed in the treatment of GDM. Glibenclamide is a second generation sulphonylurea which increases insulin release from the pancreatic β -cells. It differs from the other sulphonylureas in the high protein binding (99.8%) and short elimination half life, resulting in very low transplacental transport (170-172). All published data up to date, agree that glibenclamide is non inferior to insulin treatment in achieving desired glycaemic control. Controversy remains regarding the safety profile for the fetus. A meta-analysis (173) showed no difference in macrosomia, birth weight, LGA and neonatal hypoglycaemia compared to insulin. On the other hand, Balsells et al. comparing glibenclamide to metformin and insulin revealed higher incidence of neonatal hypoglycaemia and macrosomia in the earlier group (174).

Metformin belongs to the biguanid class. It inhibits hepatic gluconeogenesis and increases insulin sensitivity in peripheral tissues. It is well described that metformin crosses the placental barrier exposing the fetus to levels similar to maternal circulation (175). RCTs with insulin revealed higher rates of preterm births and less maternal weight gain in the metformin group. Neonatal anthropometrics were similar between treatment arms (176). Metformin is better received by patients but is associated with increased treatment failure rates, with the majority of mothers requiring the addition of insulin to achieve normoglycaemia (177). The increased transplacental transport of metformin should be considered with caution, as intrauterine programming and developmental effects on fetus have not been studied extensively. Follow up studies from the MiG cohort up to 9 years of age have not revealed any differences in BMI, body composition, metabolic health markers and neurodevelopmental progress between the 2 groups (178, 179). Similar results were presented from RCTs in pregnant mothers with PCOS. Metformin treatment during pregnancy did not affect motor social development in infancy and did not alter offspring's body composition at 9 years of age compared to the control group (180, 181).

1.4.4.1.8 Benefits of treating Gestational Diabetes

Convincing evidence suggests that treatment of GDM improves neonatal outcomes. Two RCTs comparing the effect of standard antenatal care (control) versus intensified treatment with diet, exercise and insulin (intervention) in cases of mild GDM revealed that the later approach resulted in lower birth weight and macrosomia, lower neonatal fat mass and lower incidence of CS and SD (121, 182). Whether benefits persist beyond infancy is yet to be identified. Hillier et al (155) in a cohort of 9,439 offspring showed that GDM treatment reduces risk of neonatal macrosomia and obesity at 7 years of age compared to untreated cases. Applying regression models revealed that the effect on future obesity is independent of the effect on size at birth. In contrast, following offspring from the ACHOIS trial (n=199) revealed that treatment of GDM has no effect on BMI at 4 years of age (183). Landon et al (184) showed that BMI and indices of metabolic health (blood pressure, cholesterol, insulin resistance) at 10 years of age are similar between treated and untreated cases of GDM. Inclusion of only mild GDM cases and follow up to 10 years of age are limitations of the latest studies. Inclusion of cases with severe hyperglycaemia may have led to more pronounced effect on future metabolic health, an effect which may not be apparent by 10 years of age.

Overall, the above data confirm that GDM is a complex condition leading to adverse metabolic profile via various pathways. Improving maternal glycaemic profile has significant benefits on neonatal outcomes but the adverse metabolic programming may not be related to hyperglycaemia only. Maternal BMI, GWG and biomarkers such as cytokines and lipids may be responsible for an adverse in utero environment leading to long term detrimental outcomes. In order to change the natural history of the disease, GDM pregnancies require a holistic multidisciplinary approach addressing all these risk factors.

1.4.4.2 Maternal weight - BMI

The prevalence of obesity in pregnant women follows upward trajectories in parallel with those of general population. Maternal nutritional state is one of the key determinants of offspring's future metabolic health (147). Neonatal fat mass is strongly related to maternal fat mass (185) and offspring of mothers with overweight have higher fat mass compared to those of normal weight (186, 187). The adverse metabolic programming starts from the antenatal period, as high maternal BMI is related to fetal adiposity and insulin resistance assessed by the homeostasis model (88). On the short term, maternal obesity is related to increased risk of miscarriages and congenital anomalies, stillbirth,

venous thromboembolism, maternal infections and caesarian sections. Of note, high maternal BMI predisposes to the development of GDM, pre-eclampsia and neonatal macrosomia (188). Researchers have previously focused on whether these adverse outcomes are sustained in childhood and adulthood.

Macrosomia and higher birthweight are strongly related to higher BMI in childhood and adulthood (189). Observational studies have provided evidence for the link between maternal obesity and higher offspring BMI. A large US cohort (190) studying a total of 11,120 subjects, revealed that childhood obesity rates were two and four times higher if mother was overweight or obese pre-pregnancy respectively. Similar results were presented by the UK ALSPAC group (191) after studying children up to 7 years of age. Assessing body composition, Mingrone et al (192) showed that offspring of mothers with obesity have greater fat mass at 24 years of age. Furthermore, maternal obesity is related to increased risk of offspring hypertension and cardiometabolic dysfunction, type 2 diabetes and neurodevelopmental disorders (187, 193, 194). Many researchers have argued that the associations revealed by observational studies are unlikely to be causal and may be biased by confounders. Feeding mode (women with obesity are less likely to breastfeed (195), rapid postnatal growth and maternal environment and sociodemographics may be potential contributors to the results. In order to overcome the problem, animal models have been used allowing for practicality of the research and translatability to humans. Diet induced maternal obesity in rodents programs offspring's obesity, cardiometabolic dysfunction and impaired glucose tolerance (196). Data from the Scotland national diabetes register has been recently reviewed and revealed that maternal obesity more than triples the risk of child type 2 diabetes mellitus (197).

The mechanisms underpinning the developmental programming of obesity are summarized in Figure 1.5. The maternal obesogenic environment leads to high fetal and neonatal levels of insulin, leptin, inflammatory cytokines and free fatty acids resulting in altered hypothalamic development. The imbalance between orexigenic and anorexigenic neurons and the impaired response to leptin lead to hyperphagia and obesity. The fetal exposure to hyperglycaemia and hyperinsulinaemia causes altered peripheral insulin signaling and insulin secretion resulting in impaired glucose tolerance and diabetes in future life (198). Finally, early life hyperleptinaemia may explain the development of cardiovascular and neurodevelopmental disease. Specifically, high leptin levels found in offspring of obese mothers drive sympathetic hyperstimulation leading to hypertension and vascular dysfunction and also alter maturation of hippocampus leading to impaired cognition and memory (147).

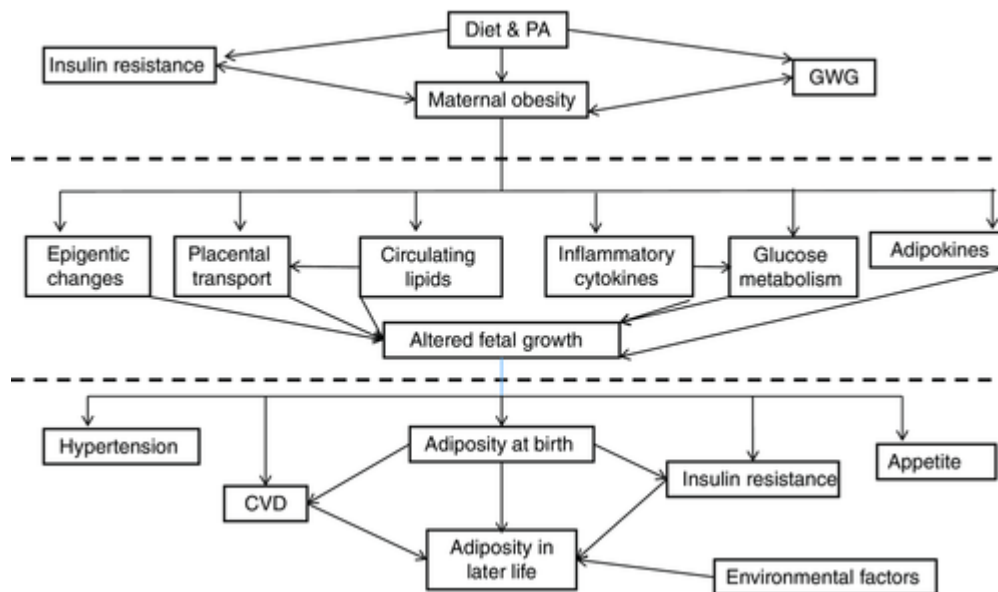


Figure 1.5: Developmental programming of obesity. Maternal insulin resistance, hyperglycaemia and dyslipidaemia lead to adverse fetal programming through various mechanisms; Adopted from “Clin Endocrinology, 2013;78-9-16”

1.4.4.3 Gestational Weight Gain (GWG)

GWG is crucial for the development and viability of the fetus (Figure 1.6). The amount of total weight gain varies depending on the number of fetuses and maternal BMI (199). GWG above the IOM recommendations is also linked to adverse metabolic programming but strength of the effect is less than that of maternal BMI. The weak association described between GWG and caesarian section, GDM and hypertension during pregnancy is more likely due to the predominant influence of BMI than weight gain per se (188). Mothers with excessive weight gain during pregnancy are at high risk of retaining weight up to 3 years postpartum which is more likely to affect subsequent pregnancies (200). On the long term, Oken et al (201, 202) studying participants from two large cohorts (Project Viva, Growing Up Today) showed that increased GWG is positively related to BMI at 3 and 14 years of age. Consistent with these results, Mamun et al (203) extended this correlation up to 21 years of age.

Improving maternal health in the peri-conceptional period is an attractive target as it would improve obesity rates for the next generations. In animal models, diet during pregnancy and lactation normalizes neonatal levels of leptin and insulin, reverses adverse programming of hypothalamus resulting in less hyperphagia and future obesity (204). In humans, siblings born before and after mother underwent bariatric surgery to achieve weight loss were studied, allowing adjustment for genetic factors. Offspring born

post surgery have lower rates of macrosomia, type 2 diabetes, obesity and hypertension (205, 206). Muktabhant et al (207) showed that a combination of diet and exercise reduces GWG and macrosomia without providing evidence about potential effects on childhood obesity. The UPBEAT trial (208) studying a cohort of 1500 pregnancies complicated by obesity in the UK revealed that intense behavioural interventions lead to reduced GWG and offspring adiposity at 6 months of age. In contrast, Lifestyle in Pregnancy study (209), after randomizing 300 women with obesity to intervention versus standard care showed that reduction of GWG is not related to neonatal macrosomia or other perinatal outcomes. These results need to be considered carefully as study failed to achieve expected weight difference between the two groups. Furthermore, as maternal BMI has a greater effect on neonatal outcomes (210), it could be speculated that mothers with obesity have metabolically altered intrauterine environment from the early stages of pregnancy and intrapartum interventions have only partial epigenetic effect.

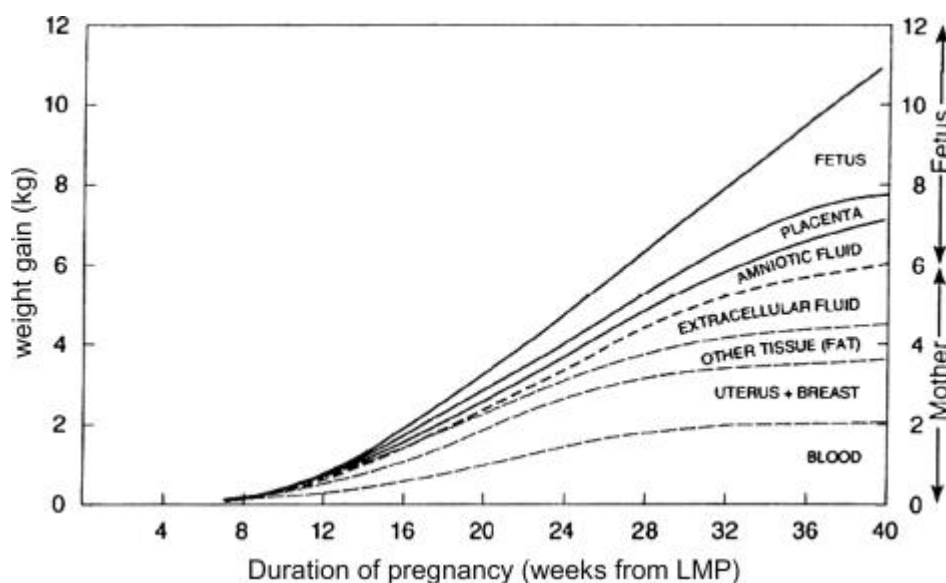


Figure 1.6: Components of gestational weight gain. Part of the weight gain is attributed to fetal weight and fluid retention; Adopted from Pitkin et al (211)

1.4.4.4 Pre-eclampsia

Pre-eclampsia is defined as hypertension (systolic blood pressure $>140\text{mmHg}$ or diastolic $>90\text{mmHg}$) and proteinuria ($>0.3\text{gr}$ in 24hr) developed after 20 weeks gestation (212). It complicates 6-10% of pregnancies, with higher numbers noted in underdeveloped countries. Maternal diabetes, hypertension, chronic kidney disease as

well as maternal age, BMI and previous history of pre-eclampsia are known risk factors (213). The pathogenesis of pre-eclampsia remains unclear. Impaired uteroplacental perfusion leads to maternal vascular dysfunction and hyperpermeability, eventually causing hypertension (214). This is related to increased fetal morbidity and mortality associated with prematurity secondary to placental insufficiency compromising fetal growth. Half of women with severe pre-eclampsia will deliver before 36 weeks gestation (212). The increased risk of childhood hypertension, obesity and diabetes is so far linked to prematurity and SGA (215) seen in pregnancies complicated with pre-eclampsia. It is well described that even increases in blood pressure (systolic or diastolic) not meeting the criteria for hypertension diagnosis are enough to increase the risk for SGA pregnancies (216, 217).

1.4.4.5 Role of Ethnicity

Some ethnic groups, in particular South Asians (SA), are at higher risk of GDM, type 2 diabetes and cardiovascular disease (218, 219). This adverse metabolic profile is also seen in the SA migrant population around the world (220). SA are known to have higher body fat content and lower muscle mass for a given body weight/BMI compared to White Europeans (221), a phenotype which is present from childhood (222). They are also known to have higher central and visceral obesity (223). Higher body fat, lower muscle mass and higher central/visceral obesity have been linked to higher risk of metabolic disorders and cardiovascular disease in any ethnic group (224).

Accumulating evidence now confirms that the adverse metabolic phenotype is present even at birth (219, 225), highlighting the role of intra-uterine programming. Fetal malnutrition, noted in SA populations, is causing redistribution of blood to brain at the expense of other abdominal organs and muscle. Fetal metabolic rate is reduced and IUGR is noted when all fetal substrates are consumed. Nutritional restriction also causes reduced Insulin-like Growth Factor (IGF) leading to further fetal growth retardation and increased cortisol levels resulting in fat accumulation (215). During fetal undernutrition, fat development is preserved as it is crucial for energy storage, thermoinsulation and providing precursors for brain development (226, 227). According to the 'thrifty phenotype' theory (228), intrauterine environment prepares the fetus for the outside world. If world and intrauterine conditions match, this association works as an adaptive mechanism. The food surplus of our modern world though, converts this association to harmful as high caloric intake is not processed properly and is converted to FM rather than FFM by the offspring, as already described above.

Yajnik et al in 2003 (229) was the first to compare infant body composition between Indians and Caucasians. His study revealed that babies born in Pune, India, despite being smaller and thinner have more adipose tissue compared to white British babies born in Southampton, UK. Since then, several studies using different methods of assessing body composition (skinfold thickness, MRI) have confirmed the “thin but fat” phenotype of SA babies which is characterized by LBW, small abdominal viscera and muscle mass but preserved fat tissue which is predominantly centrally distributed (225, 230). Trying to identify the exact mechanism underpinning this adverse metabolic programming researchers studied infants of SA origin born in continents other than Asia. Steijn et al (231) compared 5th generation SA born in Surinam, South America, with the population studied in the Southampton-Pune group. Having a homogenous genetic pool, found that the “thin but fat” phenotype is still evident but the difference in adiposity compared to white Europeans is attenuated, driven by a higher maternal BMI when compared to the Pune population. Stanfield et al (232) compared body composition at 8 weeks postpartum between infants of SA and European ancestry born in UK. After studying 30 infants in each group they reported that SA infants have lower birthweight and FFM but increased total FM compared to white Europeans. Of note, SA babies had increased subscapular (SS) and similar triceps (TR) skinfold thickness, indicating that ethnic origin predisposes to central but not subcutaneous fat accumulation. Analyzing further the previous results, SA mothers were found to be more adipose with higher rates of vegetarianism compared to controls. Recently, B12 deficiency secondary to vegetarianism was strongly linked to the development of gestational diabetes and fetal macrosomia (233). Thus, this adverse transgenerational effect characterizing SA could be due to genetics, maternal physiology and nutritional status. Identifying the exact factors responsible for this adverse programming will determine how early interventions targeting neonatal body composition need to be initiated.

1.4.4.6 Psychological causes

Maternal psychological well-being and physical activity status could potentially affect offspring's metabolic risk. Sukumar et al (data presented in DOHaD 2017) has shown that maternal depression is related to sedentary lifestyle, which in turn results in higher maternal weight gain during pregnancy, higher incidence of gestational diabetes and offspring's obesity. More studies will be required to assess the effect of these maternal characteristics on neonatal body composition and their role in programming future disease.

1.4.5 Prematurity

Preterm or premature birth is a birth of a baby born less than 37 weeks gestation. It may be spontaneous or iatrogenic, to avoid maternal/fetal complications. Highest prevalence is seen in developing countries, due to infections complicating pregnancy, but increasing rates are now noted in developed countries secondary to assisted reproduction (234). Babies born preterm may be Appropriate for Gestation Age (AGA) or Small for Gestational Age (SGA). Prematurity is linked to developmental (respiratory, gastrointestinal, visual and auditory) and behavioural problems (autism) (235) (210) with higher mortality noted in pregnancies delivered before 32 weeks of gestation. Most of the published papers study the association between low birth weight and adverse metabolic profile in the future, without providing data to assess if prematurity (duration of gestation) is an independent risk factor. Since 1990, advances in medical practice have allowed the survival of very preterm infants who are now forming a generation of adults, allowing for retrospective analysis in order to assess the association between prematurity and future diseases.

Preterm infants have low FM at birth as adipose tissue accumulates during the third trimester in pregnancy (236). At term equivalent age, preterm infants although being shorter and lighter have higher percentage of body fat compared to term (237). Of note, there is an increase in the intra-abdominal (visceral) fat whereas subcutaneous fat is reduced (238). It remains unclear if the accelerated fat deposition is secondary to prematurity, lower birth weight or “catch up” growth and the mechanisms underpinning this association are yet to be unraveled. There is no consensus regarding the body composition of preterm infants in late infancy and childhood (235). Methods of assessing body composition, quality and composition of nutrition, lifestyle and small sample numbers are potential confounders for the inconsistent results. On the other hand, researchers agree that adults born preterm have higher FM, distributed predominantly in the abdominal area, when compared to controls (239). Premature infants have an early stimulation of the hypothalamic – pituitary –adrenal axis leading to altered response to stress and excessive cortisol secretion. The hypercortisolaemia causes increased food intake and new fat deposition resulting in future obesity (240).

Prematurity is also linked to hypertension in childhood and adulthood (241). Abnormal structure of the glomeruli and less functional nephrons seen in premature babies can potentially explain the association (242). There is convincing evidence linking low birth

weight to future diabetes, but data on preterm babies remain scarce. Hofman et al (243) showed that preterm babies have less insulin sensitivity at 4 years of age when compared to term. Impaired β - cell function and insulin signaling to key peripheral tissues are known contributing factors. Finally, available literature suggests that the association between preterm birth and insulin resistance in adults exists only when rapid “catch up” growth is present (244, 245). Overall, there is accumulating evidence that prematurity is linked to adverse metabolic profile but mechanisms underpinning this association are yet to be identified. Longitudinal studies will provide knowledge on the pathophysiology of preterm birth allowing the development of strategies to amend this effect.

1.4.6 Small for gestational age (SGA) and Low Birth Weight (LBW)

SGA and LBW are terms sometimes used interchangeably in literature. LBW is defined as birth weight <2.5kg regardless of gestational age. It can be caused by fetal growth restriction (full term) or preterm delivery. SGA is weight <10th centile for a specific age and gender. Potential causes are poor maternal nutrition, infection, smoking and alcohol intake. Both SGA and LBW are associated with adverse metabolic profile in the future. Hales and Barker in 1992 (246), were the first to describe the “thrifty phenotype hypothesis”. They proposed that poor intrauterine environment leads to fetal metabolic adaptations that would allow the baby to survive postnatally in conditions of nutritional deprivation. However, if the environment provides nutritional excess, these adaptations will have long lasting detrimental effects on health.

Epidemiological evidence from Europe, America and India links LBW to metabolic syndrome and coronary artery disease in adulthood (247, 248). Longitudinal studies in different ethnic groups have shown a "U" shaped association, with increased risk of developing type 2 diabetes and obesity at both ends of the birth weight distribution (<2.5kg and >4kg). (249). UK cohorts have shown that LBW has a stronger effect on future obesity compared to macrosomia. Specifically, LBW babies have seven and fourteen times higher risk of developing type 2 diabetes and metabolic syndrome respectively, when compared to macrosomic (86, 250). Furthermore, allowing for the control of maternal and genetic factors, studies on monozygotic twins have shown that the smaller twin tends to have greater susceptibility to metabolic disease (251).

Evidence from animal models has shed light to the mechanism underpinning these correlations, with leptin having a key role in the development of future obesity. Both obese (252) and growth restricted pups (253) have altered postnatal leptin surge leading

to impaired development of the hypothalamus. As a consequence, offsprings develop hyperphagia and obesity as adults. Timing of maternal undernutrition is also critical. Nutrient restriction during early pregnancy results in future adiposity (99) whereas undernutrition during late stages of pregnancy causes a leaner phenotype (100). These findings are consistent with the Dutch Hunger Winter study (101), where exposure to famine in different periods during pregnancy had varying outcomes. Early growth restriction in rat models results in altered tissue growth aiming to preserve the brain development at the expense of other organs (254). As a result, β -cell mass (pancreas), muscle mass and glomeruli (kidney) are reduced leading to future impaired glucose tolerance and hypertension respectively (92, 94, 255).

What is not clear from current literature is if this increased risk of adverse developmental programming is directly associated to the size at birth or is due to “catch up growth”. Both SGA and LBW babies will have a ‘catch up growth’, a compensatory growth following a period of slower development. This ‘catch up’ takes place over 6-18 months of life with 85% of babies completing it by 2 years of age (256). There is strong evidence that ‘catch up’ growth reduces hospital admissions, neurodevelopmental delay incidence and risk of short stature (257). On the other hand, rapid weight gain and crossing growth percentiles in early infancy is related to increased adiposity and insulin resistance in childhood and adulthood (258, 259). Tzoulaki et al (260) showed that increased weight velocity during the first 2 years is related to hypertension and increased BMI at 30 years of age. SGA have lower levels of leptin compared to AGA babies, due to lower total FM. Lower leptin levels predispose to hyperphagia and rapid weight gain in the immediate postnatal period (261). During recovery from malnutrition offspring’s FM accumulates faster than muscle mass (262) leading to increased abdominal adipose tissue which persists through adulthood. It is therefore essential to achieve a “healthy catch-up growth”, a balance between adequate growth and normal body composition. Close nutritional management and regular monitoring of offspring’s body composition will prevent the development of future adverse metabolic profile.

1.4.7 Feeding pattern

Cesar Victora wrote that “breastfeeding (BF) is the most personalized medicine an infant can receive; an opportunity for health imprinting that should not be missed” (263). WHO recommends 6 months of exclusive BF, expanded to 2 years with additional food supplementation (264). Globally 38% of infants are fed as per WHO recommendations, with suboptimal feeding contributing to 800,000 infant deaths. Prevalence of BF by 12

months is less than 20% in high income countries, with UK having one of the lowest rates of <1% (260). In 2015, only 30% of UK born infants were exclusively BF at 6-8 weeks postpartum (265). Low maternal education, practicality, cultural and family beliefs on BF are known factors leading to such low levels. UK government and health services have been trying to increase BF rates by introducing national strategies which include structured postnatal support, regular Health Visitor visits and BF facilities in all public places (262).

BF also has a protective role for mothers, as it reduces risk for breast (266) and ovarian cancer (267). Evidence on diabetes prevention remains weaker. The short term effects of BF on neonatal well-being are well described. It is linked with a reduction in gastrointestinal, respiratory, ear infections and hospitalization from infectious causes (268, 269). In the long term, BF prevents the development of non-communicable diseases. Despite controversial evidence in the past, several meta-analyses in the last few years have shown that breastfed infants have a lower risk of developing overweight and obesity in childhood (270, 271). Furthermore, this risk is also associated with the duration of BF as there is a 4% reduction for each month of BF (272). In the ALSPAC UK cohort, BF was related to slower weight gain and lower BMI up to 5 years of age (273). Weight gain and growth velocity in early infancy are negatively related to future obesity, as described before (258). Available studies have not revealed any association with future blood pressure and cholesterol levels (270).

Many potential pathways involved in the beneficial effect of BF have been suggested. Postnatal nutrition and environment is equal important to in utero in programming future metabolic disease. Formula milk contains more energy, protein and micronutrients compared to breast milk (274). The higher caloric intake of formula fed infants leads to increased insulin secretion resulting in higher weight gain and growth velocity in early infancy, a strong indicator of future obesity. Breast milk contains many active hormones and peptides which may be crucial for neonatal health. Ghrelin and adiponectin are hormones present in breast milk which could potentially affect satiety and energy expenditure, but up to date leptin is the most well described bioactive component (275). Leptin is present in maternal (276) but not formula milk (277). In humans, leptin concentration in breast milk varies between people and is positively correlated to maternal plasma levels and maternal adiposity (276). Rat models confirm that leptin from maternal milk can be absorbed by the immature stomach epithelium to the infant circulation (278). Miralles et al (279) after studying 28 non-obese mothers showed that milk leptin levels at 1 month negatively predict BMI at 2 years of age, suggesting that the early stages of lactation are critical for offspring's phenotype. The above observation can

be explained by the leptin's role on regulating appetite and energy expenditure. Moreover, various studies have suggested that breastfed infants have better self regulation of food intake both when BF and eating solids (280).

In summary, early growth rate is a drive to compensate for adverse intrauterine environment. Formula milk lacks leptin and provides higher caloric load, leading to increased weight gain in early infancy and eventually childhood obesity. It would be of great interest to assess the effects of breast versus formula feeding on body composition during the first months of life using direct measures of adiposity. This would allow a better understanding of the mechanisms underpinning the beneficial effects of BF on future metabolic health.

1.5 Adipose tissue: A complex cell system

1.5.1 Function and types

Adipose tissue is a loose connective tissue providing structural and metabolic support. It acts as the body's fuel reservoir, storing energy in times of excess and providing fuel during fasting. It is the primary site for lipid mobilization and thermoregulation. Adipose tissue is also the largest endocrine organ. Through endocrine, exocrine and autocrine action adipose tissue regulates appetite, energy expenditure, metabolism, fertility and immunity by targeting organs as the hypothalamus, pancreas, liver, kidneys and muscle (281). Finally it is strategically placed in areas such as palms, soles, orbits, to provide mechanical support and protect from the impact of shock.

Lipogenesis and lipolysis are the 2 main processes taking place in the adipocyte throughout life. A fine balance between these two, secures energy homeostasis and insulin sensitivity. During lipogenesis excess energy is stored in the form of triglycerides in the adipocyte. After a meal, triglycerides circulate in the form of Very Low Density Lipoproteins (VLDL) and Low Density Proteins (LDL). Via the lipoprotein lipase action, lipoproteins are degraded and Free Fatty Acids (FFA) are released and enter the adipocyte. The glycaemic component of the meal, is converted to glycerol, enabling FFA esterification to form triglycerides (282). During fasting, stress hormones like adrenaline and glucagon are secreted and insulin levels drop. This results in the activation of lipase breaking down triglycerides to FFA and glycerol. FFA travel bound to albumin to other organs for oxidization and energy production. Glycerol is absorbed from liver for either oxidization or gluconeogenesis (Figure 1.7) (283).

In periods of prolonged positive energy balance, the excessive energy stored in the form of triglycerides leads to increased droplet size and lipid cell hypertrophy. When the storage capacity of the adipocyte is saturated, cells respond with continuous lipolysis providing excessive amounts of glycerol and FFA to the circulation, leading to ectopic fat tissue deposition. The ectopic lipid accumulation, mainly affecting the liver (steatosis) and muscles, results in increased insulin resistance, the main risk factor for metabolic syndrome and cardiovascular disease (Figure 1.8) (284).

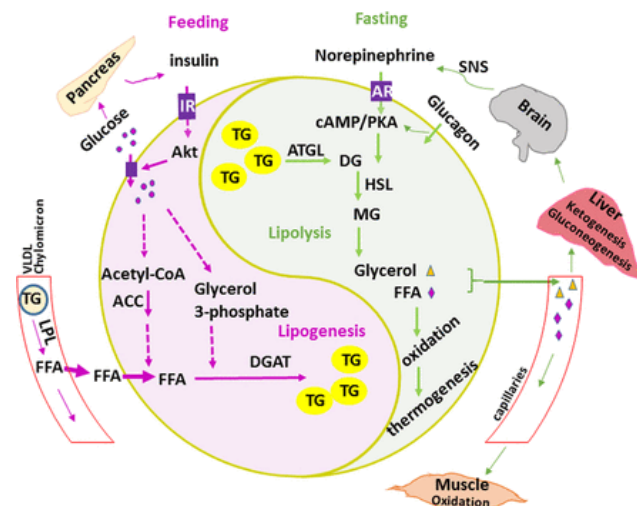


Figure 1.7: Lipogenesis and Lipolysis; Adopted from Luo et al (1)

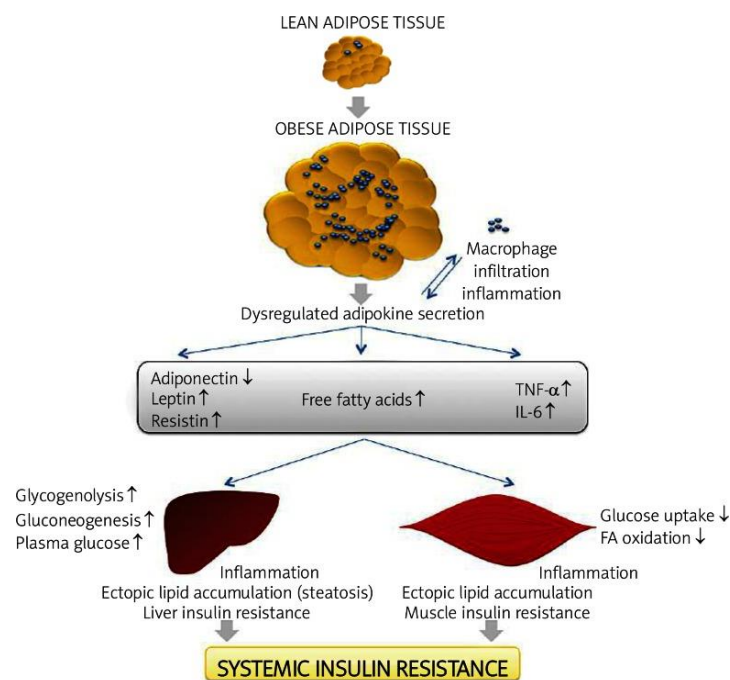


Figure 1.8: Effects of adipocyte hypertrophy in obesity. The low grade inflammation leads to impaired secretion of adipocytokines resulting into ectopic fat deposition and insulin resistance. Adopted from Coelho et al (281)

Adipose tissue formation starts in the antenatal period and continues throughout life. It consists of stem cells (or pre-adipocytes), adipocytes and the stroma vascular fraction, which contains mural, endothelial, neuronal and blood cells. Anatomically adipose tissue can be found subcutaneously, viscerally, in the bone marrow, between muscles and in the breast. Various adipose depots have discrete morphological and functional characteristics. The role of intrauterine programming in the development and distribution of adipose tissue has drawn the attention of research activity in the last decades (285).

There are 3 types of adipose tissue, characterized by different origin, morphology and mitochondria abundance: White Adipose tissue (WAT), Brown Adipose Tissue (BAT) and Beige (brown in white).

1.5.1.1 White Adipose Tissue

WAT arises from mesenchymal progenitors and its main role is to store (in the form of triglycerides) or supply energy (through free fatty acid and glycerol formation) when required. Plays a key role in body metabolism and excess (obesity) or paucity (lipodystrophy) of WAT is related to metabolic abnormalities (impaired glucose metabolism), hypertension and carcinogenesis (286). Furthermore, presence of subcutaneous and dermis WAT maintains body temperature, acting as a thermal insulation (1).

WAT can be further categorized to subcutaneous and visceral fat, each having unique anatomic and metabolic properties. Subcutaneous depots are found mainly in the interscapular and inguinal region. They contain heterogeneous small adipocytes, with few mitochondria, resulting in low oxidative rate. High volume of interstitial tissue is a discrete characteristic of subcutaneous fat. On the other hand, visceral depots are found in the retroperitoneal, mesenteric, omental, peri-gonadal and peri-renal region and consist of homogenous, large adipocytes, with small number of mitochondria (287).

In a simplified approach, authors describe subcutaneous as “good” and visceral as “bad” fat (288). The “pear” shaped phenotype often associated with female sex is characterised by increased deposition of subcutaneous fat around the pelvic region, resulting in lower risk of cardiovascular disease (289). On the other hand, the “apple” shaped phenotype associated with male sex, with accumulation of visceral fat around the abdominal area, is linked to metabolic dysfunction (290). In support of this, Tran et al in 2008, reported reduced insulin resistance in rodents after subcutaneous fat transplantation. Similar effect was not observed after visceral fat transplantation (291). The increased lipolytic

rate and adipocyte's turnover found in subcutaneous fat may be the explanation behind this protective role (292). Furthermore, the 2 types respond differently to stimuli. Oestrogens cause increased subcutaneous fat whereas corticosteroids enhance visceral fat deposition, thus explaining the increased cardiovascular risk linked to long term endogenous or exogenous steroid exposure (293). Finally, different transcription factors and gene expression is found between various depots. Leptin, angiotensin, adiponectin, favouring metabolic health, are produced by subcutaneous fat and pro-inflammatory cytokines, like interleukin 6 (IL-6) and plasminogen activator inhibitor 1 (PAI-1) are predominantly expressed by visceral fat (285).

In summary, WAT has the capacity to store energy in the form of triglycerides in times of positive energy balance and release it in times of fasting. Metabolic syndrome and risk for cardiovascular disease is associated with total FM but the distribution of adipose tissue plays an even more important role, with subcutaneous fat being protective for the metabolic well being.

1.5.1.2 Brown Adipose Tissue

Brown fat cells and muscle cells arise from the same stem cell population in the mesoderm (Figure 2.3). The progenitor cells carry the Myogenic factor 5 (Myf5), a trait characteristic of brown fat cells and myocytes. WAT do not activate the Myf5 (294). Anatomically, brown adipocytes are smaller with variable size lipid droplets. The presence of large numbers of mitochondria explains the increased oxidation rate reported. The brown colour is due to the increased vascularization and mitochondria presence. BAT is responsible to convert nutrients to heat by fatty acid oxidation, without adenosine triphosphate (ATP) production (295).

BAT is predominantly found in neonates, in interscapular and perirenal regions. It is responsible for thermoregulation during the early stages of life. In the past it was believed that adults do not have any brown fat tissue. As recently as 2007, during investigations to trace tumor metastasis using Fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET/CT) scan, the existence of brown fat depots was demonstrated in adult humans. The main depots are lower neck and supraclavicular areas (296). Obesity is characterized by reduced brown FM. Methods (BAT transplantation) and medication to increase brown fat formation and prevent/treat obesity by converting excess calories to heat is an area of great current research interest (297).

1.5.1.3 Beige (Brown in White)

Beige adipocytes, are brown like thermogenic cells found mainly in subcutaneous and to a lesser extent visceral white fat. Despite having the same thermogenic function with BAT, they arise from Myf5 negative progenitor cells, like WAT (Figure 1.9) (298). Another theory supports that transdifferentiation of mature WAT may result in the formation of new beige cells (299).

In conclusion, BAT and beige are responsible for the non-shivering thermogenesis. Cold, food intake and stress release hormones like insulin, Glucagon Like Peptide 1 (GLP-1), leptin and triiodothyronine (T3) which stimulate the hypothalamus (300). In return, high doses of noradrenaline (NA) are released through the sympathetic nerve leading to activation of brown and beige tissue, mobilization of lipids from other depots to brown and beige, increased uptake of serum circulating triglycerides and glucose from the BAT and “conversion” of WAT to beige by uncoupling protein 1 (UCP-1) activation. The above adoptive mechanism is transient and reverses back to initial state after removal of the stimulant (Figure 1.10) (301).

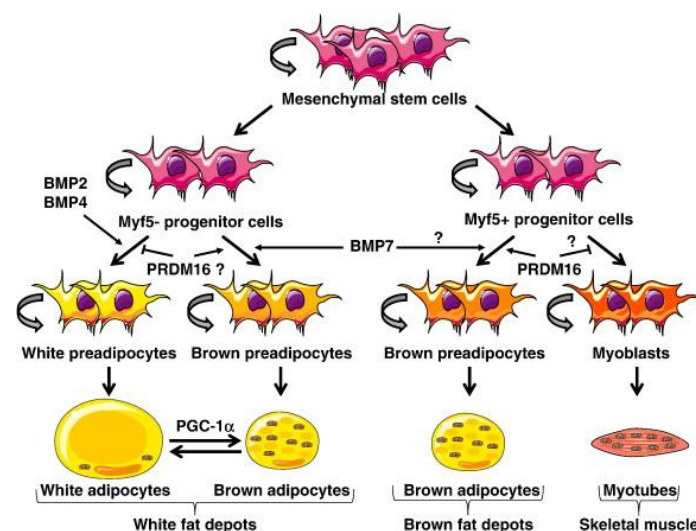


Figure 1.9: Origin of fat cells. Adipocytes and myocytes share common precursor cells; Adopted from Langin et al (294)

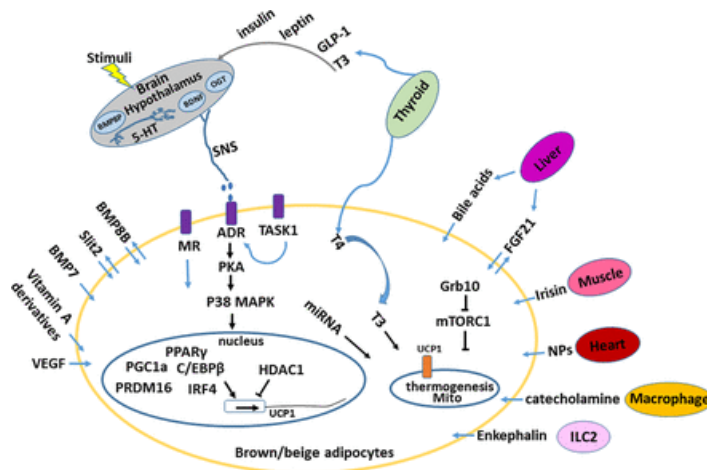


Figure 1.10: Thermogenesis; Adopted from Luo et al (1)

Adipose tissue is going through dynamic changes during life, depending on energy balance. It is able to expand in order to accommodate increased lipid demand by adipogenesis, hyperplasia and hypertrophy.

- Adipogenesis: Pre-adipocytes of mesenchymal origin convert to mature fat cells. Peroxisome proliferator activated receptor γ (PPAR γ) is the master regulator of adipogenesis and adipose tissue maintenance (302).
- Hyperplasia: Preadipocytes within adipose tissue, convert to fat cells in cases of energy excess.
- Hypertrophy: Mature fat cells can increase their size to accommodate increased storage needs

Obesogenesis is characterized by increased rate of all 3 pre-mentioned functions. Hypertrophic cells have reduced lifespan due to increased apoptosis. Macrophages responsible for clearance, contribute to this inflammatory state. Cytokines as IGF-1, Tumor Necrosis Factor α (TNF α) are released leading to altered biology of adipose tissue (imbalance between lipogenesis-lipolysis, impaired secretion of inflammatory-protective adipokines) and insulin resistance (303).

1.5.2 Adipose tissue as an endocrine organ

Adipose tissue was once considered to be fat storage only. Today WAT is described as the largest endocrine organ, synthesizing a variety of biologically active hormones (Table 1.6).

Adipocytokines	Function
Leptin	Regulates appetite and energy expenditure, Possible role in intrauterine growth
Adiponectin	Reduces hepatic gluconeogenesis, reduces insulin resistance and increase peripheral glucose uptake
Resistin	Increases insulin resistance
TNF α	Pro-inflammatory, increases insulin resistance
IL-6	Pro-inflammatory, participates in lipid and glucose metabolism
PAI-1	Inhibits plasminogen activation
Angiotensinogen	Regulator of blood pressure and electrolyte homeostasis
IGF-1	Growth hormone mediator, promotes proliferation
FFA	Oxidized to produce energy, component of triglyceride
Glycerol	Component of triglycerides and gluconeogenesis pathway

Table 1.6: Hormones produced by adipose tissue. Adipocytes produce a variety of adipocytokines with unique metabolic profile

Depending on the location of the fat depots, different adipocytokines (also known as adipokines) are secreted, with visceral fat having a more active role. Adipocytokines bind to receptors of target organs, triggering intracellular pathways. Adipose tissue itself is expressing receptors for these hormones, contributing dynamically to energy and metabolism homeostasis (304).

During obesogenesis, the low grade inflammation described before, is contributing to impaired synthesis and secretion of adipocytokines. The imbalance between protective (leptin, adiponectin) and harmful cytokines (IL-6, TNF α , PAI-1) leads to increased insulin resistance resulting in metabolic syndrome, characterized by diabetes mellitus, hypertension and dyslipidaemia (Figure 2.2) (305).

Leptin and adiponectin are 2 of the main adipocytokines related to metabolic control and will be described in detail for the purpose of this thesis.

1.5.2.1 Leptin

The discovery of leptin in 1994, established adipose tissue not only as an energy storage but also as an endocrine organ. Leptin originates from the Greek word “leptos”, which means thin. It is a 16kDa protein, consisting of 167 amino acids and produced by the Ob gene, located in human chromosome 7q31.3 (306). Studies, predominantly on mouse models, have helped understand leptin’s pathophysiology and its role in obesity. Ob/Ob (leptin deficient) and db/db (leptin resistant) mice are characterised by early life obesity, diabetes mellitus, hypothermia and infertility (307). In humans, obesity is characterized by leptin resistance rather than deficiency and cases due to Ob/Ob mutation are rare

(308). Leptin receptor defects, inhibition of leptin signaling and reduced blood brain barrier transport are possible explanation of the leptin resistance noted (309). The role of leptin in the treatment of adult obesity has been a project of extensive research, but is beyond the scope of this thesis and won't be described further (310).

Adipose tissue is the main site of leptin production. WAT is the major source, whereas BAT was recently found to contribute to a lesser extent to the synthesis. Leptin gene is expressed differently in various fat depots, with subcutaneous fat producing more leptin when compared to visceral (311). Multiple studies have identified leptin mRNA in other human tissues. Ob gene was found to be expressed in ovaries, muscle (312), mammary cells (313) and bone marrow (314). Stomach is the only gastrointestinal tissue expressing the leptin gene (315). The presence of leptin production in placenta and fetal tissues implies the role of leptin as a growth factor (316). In summary, the production of leptin in tissues other than adipose, lead to the conclusion that leptin has a more complicated role than only being a marker of energy balance and adiposity.

Leptin synthesis is affected by various factors (Table 1.7). The secretion follows a pulsatile pattern with diurnal variation. Higher levels are noted at midnight and early morning (317, 318). Obesity, overfeeding and hyperglycaemia increase leptin production. Oestrogens promote subcutaneous fat depots in contrast to the testosterone effect, leading to sex dimorphism. Females have more leptin for a given total FM compared to males. On the other hand, fasting, cold and long term exercise cause reduction of leptin production (319). In general, leptin synthesis is mediated through sympathetic nervous system; B-agonists, noradrenaline and isoprenaline inhibit leptin synthesis (320).

Factors that increase serum leptin levels	Factors that decrease serum leptin levels
Obesity	Androgens
Overfeeding	Fasting
Impaired renal function	Cold exposure
Insulin	Growth hormone
Glucose	Long term exercise
Glucocorticoids	Somatostatin
TNF- α	Thiazolidinediones
Oestrogen	Smoking
Endotoxin	IGF-1
Interleukin-1	Free fatty acids
Alcohol	β -Adrenoceptor agonists

Table 1.7: Factors affecting leptin production

Energy homeostasis is achieved through pathways that influence feeding and metabolic rate. Peripheral tissues secrete hormones which reflect adipose tissue stores and nutritional state. Adipocytokines, insulin and gut hormones act centrally at the level of hypothalamus. Specifically, the ARC is the site in the hypothalamus receiving these signals (321). It is composed by 2 types of neurons:

1. anorexigenic neurons, secreting proopiomelanocortin, cocaine and amphetamines
2. orexigenic neurons, secreting neuropeptide Y and agouti-related protein

Leptin's main role is to reflect energy reserves, regulate appetite and energy expenditure by signaling the CNS. Its action is mediated by central and peripheral receptors (ObR) (322). Centrally, in conditions of positive energy balance, increased leptin levels bind to hypothalamus which in turn releases anorexigenic and inhibits orexigenic hormones. Leptin also acts to the mesolimbic dopaminergic system and the brainstem promoting satiety (Figure 2.5). Furthermore, hypothalamus responds to the increased energy expenditure demand by producing Thyrotropin Releasing Hormone (TRH), Gonadotropin Releasing hormone (GnRH) and IGF-1, leading to increased metabolic rate (323). Leptin treated mice loose more weight and develop increased metabolic rate and core temperature than pair-fed controls, proving that leptin regulates both appetite and energy expenditure (324).

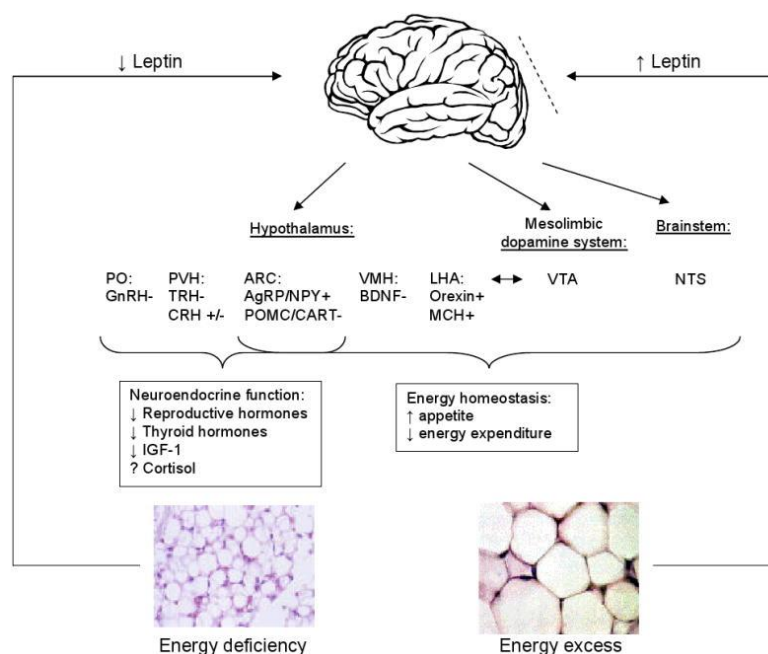


Figure 1.11: Central effects of leptin. Leptin promotes the secretion of anorexigenic hormones and increases energy expenditure through adrenergic stimulation. Leptin excess promotes LH/FSH, IGF-1 and TSH secretion; Adopted from Kelesidis et al (325)

Numerous reports have described the presence of ObR in peripheral tissues. Leptin receptors belong to the gp130 cytokine receptors family, expressing a CK-F3 domain as the binding site to leptin. Following binding, receptor degrades leading to intracellular activation signal (322). The ObR gene is expressed in various human tissues, including lungs, heart, kidney, liver, spleen, small intestine, ovaries, testis and adipose tissue (326). Placenta and multiple foetal organs also express ObR (327). Leptin which was one thought to be a marker of energy stores is now well proven to have a more complex physiology (Figure 1.12) participating in haemopoiesis, angiogenesis, reproduction and fertility, pregnancy and fetal growth, puberty and immunity (319).

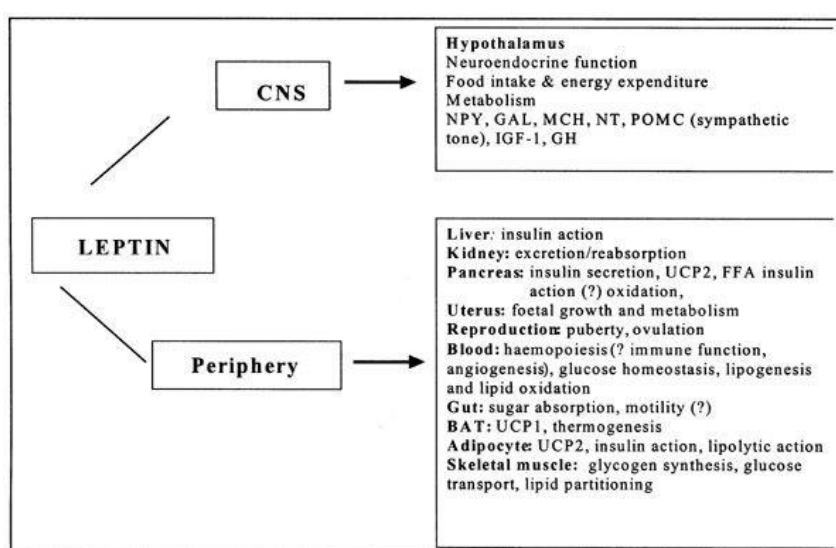


Figure 1.12: Leptin physiology. Leptin demonstrates both central (appetite regulation, sympathetomimetic) and peripheral actions (increased energy expenditure, glucose - lipid metabolism and reproduction); Adopted from Margetic et al (319)

1.5.2.1.1 Leptin and insulin/metabolism

Of great interest is the role of leptin in human metabolism. Insulin is an anabolic hormone, promoting fuel storage, in contrast to leptin which mobilizes triglycerides leading to energy expenditure through FFA oxidization. Insulin enhances leptin production acting directly to the adipocyte level. The above statement is further supported by the presence of high leptin levels in cases of insulinoma (328). On the other hand, leptin inhibits insulin secretion by pancreatic islet cells (329). The main metabolic effect is mediated through direct action to vital organs (liver, muscle, adipose tissue) causing reduced lipogenesis, reduced glycogen synthesis and increased FFA oxidization (Table 1.8).

Leptin has also other roles in reproduction (330), angiogenesis (331) and haemopoiesis . (332) which are beyond the scope of this thesis.

In vitro models	Pathway	Effect
Leptin effect		
Muscle	FFA oxidation	Stimulation
	Insulin mediated anti-oxidative and lipogenic effects	Attenuation
	Insulin mediated glucose transport	No effect
	Glycogen synthesis	No effect
Adipose	Insulin binding	Inhibition
	Insulin mediated glucose transport- glycogen synthase activity, lipogenesis	Inhibition
Liver	Insulin binding	Inhibition
	Glucagon activated cAMP production	Inhibition
Insulin effect		
Adipose	Leptin secretion and gene expression	Stimulation

Table 1.8: Leptin interactions with insulin-glucagon. Table demonstrates the effect of leptin on vital organs such as liver, muscle and adipose tissue

1.5.2.2 Adiponectin

Adiponectin is a product of the ADIPOQ gene, located in chromosome 3q27. It was first described in 1995 and during the following two years various groups had successfully studied its molecule, leading to different names such as ARCP30 (adipocyte complement related protein), AdipoQ and GBP28 (gelatin binding protein 28). Adiponectin is the most commonly used name. Structurally full length adiponectin consists of 247 amino acids, posing similarities to complement and TNF- α molecules. In detail, there are 3 distinct compartments: i) aminoterminal peptide ii) collagen like domain at N-terminal and iii) globular domain at c terminal, similar to complement and facilitating binding to adiponectin receptors (Figure 1.13) (2).

After synthesis, adiponectin undergoes hydroxylation resulting in 8 isoforms. Main structural form is a trimer, formed by the connection of 3 monomers. 2-6 trimers combine to create higher molecular weight structures (hexamers, multimers). High molecular weight (HMW) and hexamers are the main circulating forms whereas trimers have a shorter half life. <1% of circulating adiponectin is the globular-adiponectin, arising from the proteolysis of the full length molecule and corresponding to the globular domain. Similar to trimers, globular-adiponectin has a short duration of life. Multimeric forms have different biological activity, targeting various human tissues (333). HMW adiponectin poses a more peripheral action, targeting predominantly the liver and muscles, having an insulin sensitizing effect whereas lower weight isoforms tend to act centrally at the level of hypothalamus (334).

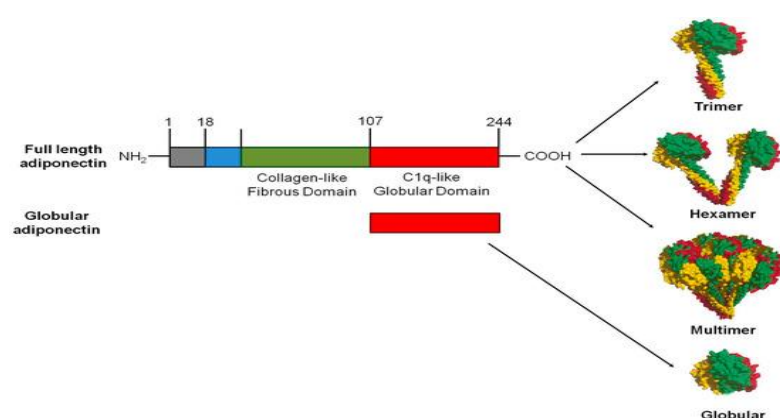


Figure 1.13: Structure of adiponectin. The aminoterminal peptide (blue), collagen like domain at N-terminal (green) and globular domain at c terminal (red) are color coded; Adopted from Achari et al (335)

Adiponectin is almost exclusively secreted by WAT in adults. Recent studies have confirmed that central nervous system, bone marrow and osteoblasts, myocytes and cardiomyocytes, salivary gland and fetal tissues are other sources of adiponectin in humans (336-338). Adiponectin production in adipose tissue is regulated at the level of gene expression (339). Post translational changes, including hydroxylation and glycosylation, define the type of oligomers in circulation (340). Adiponectin gene is a target for peroxisome proliferator activated receptor γ (PPAR γ) regulation. Activation of the receptor enhances adiponectin expression whereas TNF α , IL-6 and resistin inhibit it. PPAR is a subgroup of nuclear family receptors controlling major aspects of metabolic homeostasis. They are expressed in many human tissues but are predominantly found in WAT and BAT (341). Specifically, PPAR γ are the master regulators of adipogenesis and adipose tissue differentiation, mediating adiponectin autocrine action. They increase

lipogenesis without creating larger adipocytes, more and smaller adipose cells are created instead (342) and promote subcutaneous to visceral fat differentiation (343). Adipocyte formation is impossible without the presence of PPAR γ , which are induced when preadipocytes convert to adipose cells (344). Deletion of PPAR γ in knockout mice, leads to apoptosis of mature adipocytes and insulin resistance (345). The close relationship between PPAR γ , adiponectin production and insulin resistance has led many researchers during the last decades in the study of substrates which could potentially activate the receptors, thus improving insulin sensitivity and metabolic profile. Thiazolidinediones (TZD), medications used in diabetes mellitus, are proven to be direct agonists of the receptors and will be discussed later in this chapter.

Adiponectin action is mediated through 3 different receptors (Figure 1.14):

1. AdipoR1: Transmembrane receptors, encoded by the 1p36.13-q41 gene which are mainly expressed in human skeletal muscle. They have high affinity for gAdiponectin and less for full length isoforms (346). In mice models they participate in energy metabolism, as R1 $-/-$ mice are obese with low energy expenditure (347).
2. AdipoR2: Transmembrane receptors encoded by the 12p13.31 gene, 67% homologous with R1 but having different N-terminal sequence. They are mainly expressed in liver but also in muscles, showing great affinity for both globular and full length adiponectin (346).
3. T cadherin: Contradicting evidence exists on whether they are receptors based on the surface of the cell membrane or just binding proteins to adiponectin. They have no affinity for trimeric and globular-adiponectin but bind to HMW and hexameric adiponectin. They are encoded by the CDH13 gene and are mainly expressed in the cardiovascular system, including heart, aorta, carotids, iliac and kidney arteries (348).

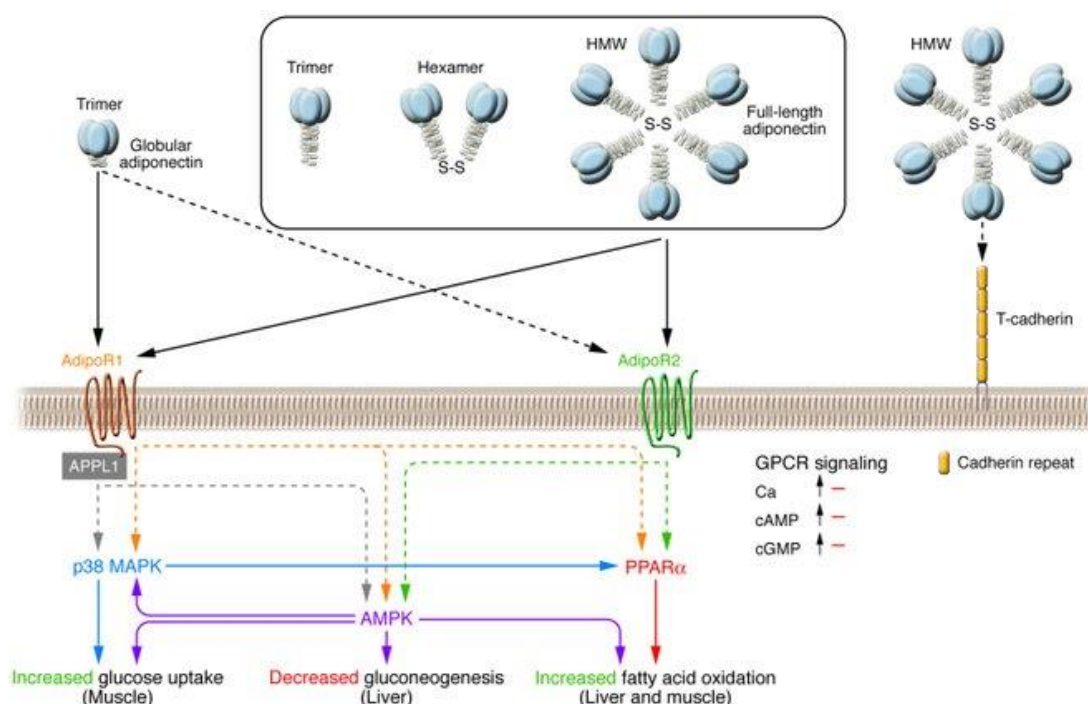


Figure 1.14: Adiponectin receptors; Intracellular signaling leads to increased lipid oxidation, increased glucose uptake and reduced gluconeogenesis Adopted from Kadowaki et al. (349)

Table 1.9 demonstrates the distribution of adiponectin receptors in various human tissues. The presence of adiponectin receptors mRNA in various organs, reveals its multifactorial role in many aspects of human physiology. The adipocytokine which was once believed to be a marker of adipose tissue is now proven to contribute to many tissue functions.

Interaction of adiponectin with its receptors triggers multiple signaling pathways. Activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK) has a primary role in the insulin sensitizing effect of adiponectin. AMPK is a cellular energy sensor, which activates energy producing pathways (fat oxidation) and inhibits energy storing (2). AMPK important role was confirmed after the suppressed fatty acid oxidation, glucose uptake and lactate production seen in dominant negative mutants (350). Adiponectin action is also mediated by other signaling molecules such as p38 mitogen-activated protein kinase (p38 MAPK), peroxisome proliferator-activated receptor-α (PPARα) and the RAS-associated protein Rab5. Recently, the discovery of the adaptor protein APPL1 shed more light to the adiponectin signaling cascade. APPL1 has 3 functional domains which include a NH₂-terminal Bin1/amphiphysin/rvs167 (BAR), a pleckstrin homology (PH) (278–377 amino acids) and a phosphotyrosine binding (PTB) domain. APPL1 binds to adiponectin receptors and mediates positively the adiponectin

signaling (Figure 1.15). APPL1 deficient models, are characterized by reduced fat oxidation, glucose uptake and AMPK phosphorylation (351).

		AdipoR1	AdipoR2	T-Cadherin
Skin-Adipose	Skin	•	•	•
	Subcutaneous	•	•	•
	Visceral	•	•	•
Muscular system	Muscle	•	•	•
Nervous system	Brain	•	•	•
	Cerebral cortex			•
	Medulla			•
	Thalamus			•
	Midbrain			•
	Hypothalamus			•
	Spinal Cord			•
Circulation	Heart	•	•	•
	Blood vessels	•	•	•
Endocrine	Adrenal gland	•	•	
	Pituitary	•	•	
	Pancreatic cells	•	•	
	WAT	•	•	
	BAT	•	•	
GI system	Stomach	•	•	
	Small Intestine	•	•	
	Large Intestine	•	•	
	Liver	•	•	
	Pancreas	•	•	
Immune system	Thymus	•	•	
	Lymph nodes	•	•	
	Bone marrow	•	•	
Respiratory	Lungs	•	•	
Urinary	Bladder	•	•	
	Kidneys	•	•	
Reproduction	Testes	•	•	
	Prostate	•	•	
	Breast	•	•	

Table 1.9: Adiponectin receptors in various human tissues. The widespread localization of adiponectin receptors suggest a crucial role in human physiology

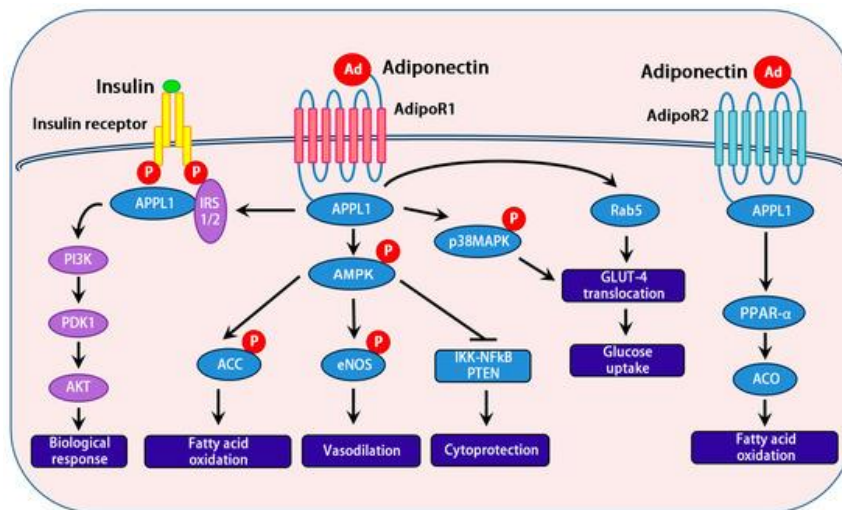


Figure 1.15: Adiponectin receptor signaling; Adopted from Achari et al (335)

1.5.2.2.1 Adiponectin and liver

Liver, the cornerstone organ of human metabolism, expresses adiponectin receptor mRNA (352). Hepatocytes express greater amount of AdipoR2 than AdipoR1. Adiponectin inhibits FFA synthesis and promotes oxidization by up regulating PPARα and AMPK mediators. It plays an important role in glucose homeostasis by suppressing glycogenolysis and gluconeogenesis and by increasing glucose uptake (353). Multiple studies have confirmed the suppressed endogenous glucose production, without hypoglycaemic episodes, after adiponectin infusion, even at supraphysiological doses (354).

Adiponectin has a protective role against liver disease. In Ob/Ob mice, adiponectin supplementation prevented fatty liver and alcohol induced steatosis. In human models, chronic hepatitis and steatosis grade were negatively related to adiponectin levels. The above effects could be explained by the ability of adiponectin to reduce acetyl-CoA carboxylase and fatty acid synthase activity (355).

1.5.2.2.2 Adiponectin and muscle

Adiponectin is expressed in human skeletal muscle (356), another crucial tissue for energy metabolism. Activating AMPK and p38 MAPK, adiponectin leads to higher glucose uptake by the muscle and FFA oxidization (357). Moreover AdipoR1 and AdipoR2 are also expressed in muscle with R1 having a more predominant role (358). Of great importance, Prior et al (359) showed that the receptor expression is inversely

related to insulin levels. Thus, in conditions of fasting or insulin deficiency, adiponectin action is promoted by increased receptor (AdipoR1) expression. Recently, the association between adiponectin and muscle growth has been addressed. Fiaschi et al (360) showed that globular adiponectin induces muscle gene expression and cell differentiation in murine cells.

1.5.2.2.3 Adiponectin and pancreas

Human pancreatic β -cells express R1 and R2 receptors. Adiponectin deficient mice have low plasma insulin after glucose loading, suggesting a direct effect of adiponectin on pancreatic insulin secretion (361). To further support this statement, Okamoto et al (362) showed that the adiponectin infusion to isolated islet cells and mice increased insulin levels when compared to placebo.

There is contradicting evidence regarding the role of adiponectin in the pathogenesis or prognosis of acute and chronic pancreatitis. Whether adiponectin protects pancreatic cell apoptosis remains to be examined further (363).

1.5.2.2.4 Adiponectin and central nervous system

The role of adipose tissue derived hormones, such as leptin, in regulating food intake and energy expenditure through CNS stimulation is well described. There is conflicting evidence regarding the role of adiponectin with some studies showing no effect on food intake and increased energy expenditure (364) whereas others revealing increased food intake and negative energy balance (350). Conflicting results also exist on whether adiponectin passes the blood-brain barrier (365). Interestingly, AdipoR1 and AdipoR2 are widely distributed in areas of CNS responsible to control food intake and energy balance, such as pituitary, hypothalamus and brainstem (366). Given the known adiponectin production in the CNS, a potential autocrine/paracrine role of adiponectin needs to be further investigated.

1.5.2.2.5 Adiponectin and diseases

Adiponectin has been related to the pathogenesis of various diseases and its role as a prognostic or therapeutic marker is the area of current research. In detail:

1.5.2.2.6 Adiponectin and inflammatory state

Adiponectin exerts anti-inflammatory effects, which differ according to adiponectin isoforms and target cells. In vitro studies, adiponectin increased anti-inflammatory cytokines (IL-10) and reduced IL-6 and TNF- α production from macrophages (367). Tsatsanis et al (368) showed that macrophages' immediate response to adiponectin exposure is to increase pro-inflammatory cytokines production. However, prolonged exposure leads to inhibition of further production, suggesting a form of resistance and adaptation. Finally, chronic inflammatory states, such as obesity, characterized by persistent TNF- α production by adipocytes, leads to reduced adiponectin expression (369).

1.5.2.2.7 Adiponectin and obesity

Measures of adiposity such as BMI, waist circumference and abdominal fat are negatively related to serum adiponectin levels (370). Despite adiponectin secretion from adipose tissue, humans with obesity have lower levels compared to lean (371). A possible explanation to this paradoxical observation is that the hyperplastic adipocytes seen in obesity secrete hormones like TNF- α and IL-6 which suppress adiponectin production. Even the expression of AdipoR1 and AdipoR2 is downregulated in cases of obesity (372). It looks like adiponectin has an autocrine role with negative feedback on its own production. The question of whether adiponectin is a "cause or consequence" of obesity remains to be answered.

1.5.2.2.8 Adiponectin and diabetes mellitus

Adiponectin exhibits its beneficial role on glucose metabolism through AMPK and PPAR α activation, leading to reduced gluconeogenesis and increased glucose uptake (349). In hyperinsulinaemic-euglycaemic clamps, adiponectin negatively predicts the degree of insulin resistance, confirming its insulin sensitizing role (373).

PPAR γ is a major regulator of adipocyte gene expression and adipocyte differentiation. Negative PPAR- γ mutations are related to severe insulin resistance, development of type 2 diabetes mellitus and fivefold reduction in circulating adiponectin levels (374). TZDs improve glucose tolerance by activation of PPAR- γ receptors. Administration of TZDs in insulin resistant humans has increased serum adiponectin levels by promoting expression of adiponectin gene, mediated by PPAR- γ activation (375). On the other hand, adiponectin deficient mice, develop impaired response to TZD treatment, implying

that the benefits of the treatment are mediated by increased adiponectin production. Muscle and liver express less numbers of PPAR γ compared to adipose tissue, therefore the glucose utilizing effect of TZD is indirect through adiponectin production (Figure 1.16).

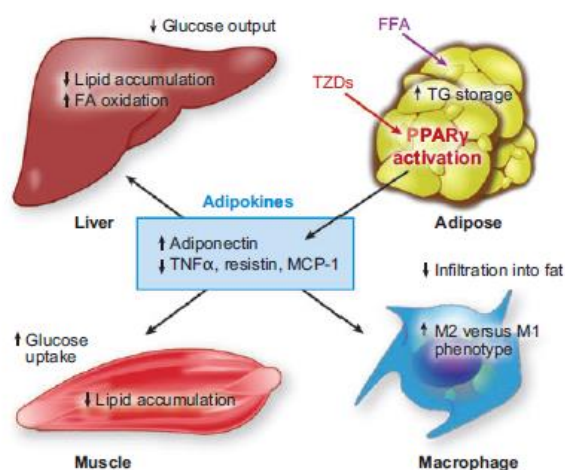


Figure 1.16: Activation of PPAR γ by thiazolidenidiones (TZD). PPAR γ activations leads to favorable metabolic effects; Adopted from Maeda et al (375)

Multiple studies have shown that patients with diabetes have lower adiponectin levels (376). Both total and HMW adiponectin are negatively correlated with glucose and insulin levels (377). The mechanism responsible for the low adiponectin levels in insulin resistant states is still unclear. These observations led to the question of whether hypoadiponectinaemia is a cause or a consequence of insulin resistance. Yamauchi et al in 2001 (378), studied lipoatrophic mice with absent adiponectin and noted that adiponectin infusion corrected hyperinsulinaemia and hyperglycaemia. Moreover, Kubota et al (379) showed that despite adiponectin knockout mice have the same insulin levels with controls, the glucose effect is much lower, confirming the role of adiponectin as insulin sensitizer. Subjects with genetic insulin receptor deficiencies, characterized by insulin resistance, have higher adiponectin levels (380). On the other hand, exogenous insulin infusion is shown to reduce adiponectin levels, questioning the cause-effect link between adiponectin and insulin resistance (381).

In an attempt to answer the previous question, Li et al (382) performed a systematic review including thirteen prospective observational studies (n= 14,598 participants). Results of the meta-analysis revealed that higher adiponectin levels can predict reduced risk of future type 2 diabetes mellitus. One of the main disadvantages of his work is that observational studies cannot prove causality and results can be biased by many

covariates. In order to provide unbiased results Yaghootkar et al (383) performed a large Mendelian randomization study. Purpose of the Mendelian randomization was to use genetic polymorphisms with well-understood effects on exposure patterns, allowing safe causal effect conclusions from observational data, in the presence of confounders. (384) In a large cohort of 31,000 individuals, results suggested adiponectin as a marker, rather than a pathogenic factor of insulin resistance. In contrast to the above results, Gao et al (385) applying also Mendelian randomization models in Swedish population (n=942 individuals), showed a positive correlation between adiponectin and insulin sensitivity. All the above, signify that up to date, the exact pathophysiological role of adiponectin and its role in the development of insulin resistance and diabetes is not fully understood. Larger longitudinal studies, including genetic data, are required to clarify the role of this elusive molecule.

1.5.2.2.9 Adiponectin and cardiovascular disease

The link between visceral fat and increased cardiovascular disease is well established (386). The more visceral to subcutaneous fat the less the adiponectin levels, therefore an inverse correlation between cardiovascular disease and adiponectin was assumed. Patients with coronary artery disease have lower adiponectin levels compared to controls (376). Specifically it is the HMW isoforms which are reduced whereas hexameric and trimeric forms remain the same.

The above association can be explained by the anti-atherogenic role of adiponectin. In its presence, monocyte adhesion to endothelial cells is reduced, conversion of macrophages to foam cells is inhibited, binding of LDL to the sub-endothelium is limited and vasodilation through nitric oxide production is enhanced (387). Chronic inflammation, a well known risk factor for cardiovascular disease, is reduced in the presence of adiponectin through inhibition of TNF α and IL-6 production and enhancement of anti-inflammatory IL-10 expression (388).

1.5.2.2.10 Adiponectin and other diseases

Adiponectin infusion has been linked to normalization of blood pressure in rat models through reduction of renal sympathetic nerve activity (389). Low adiponectin levels have been found to be associated with low HDL (high density lipoprotein), high VLDL (very low density lipoprotein) and TRG (triglycerides) levels (376) as well as increased risk of future development of metabolic syndrome (390). Finally, hypoadiponectinaemia may

participate in carcinogenesis, as low levels have been linked to increased cell proliferation and reduced apoptosis (391).

In summary, adiponectin is a complex molecule participating in the main metabolic pathways of humans. The presence of adiponectin receptors in various tissues, confirms its multifactorial role (Figure 1.17). Up to date data cannot fully explain the contribution of adiponectin to metabolism, low grade inflammation and atherosclerosis, the three main predictors of mortality. If adiponectin is a regulator or just a marker of metabolic and cardiovascular status remains to be confirmed. Research on ways to restore the capacity of adipocytes in secreting adiponectin can serve as a potential therapeutic approach to metabolic diseases. The prognostic value of adiponectin in the development of diabetes and cardiovascular morbidity and mortality remains to be clarified by further studies and could potentially constitute a biomarker allowing for early intervention in high risk groups.

1.5.2.3 Adipocytokines in pregnancy

Insulin and IGF-1 are the most well described growth factors during pregnancy (392). As the role of intrauterine exposure in the development of future metabolic diseases is established, finding a link between fetal markers and risk of adverse metabolic profile would enable us to understand the mechanisms underlying this association. Leptin and adiponectin may play an important role in fetal growth either having a direct effect or acting as mediators and sensitizers to insulin and IGF-1.

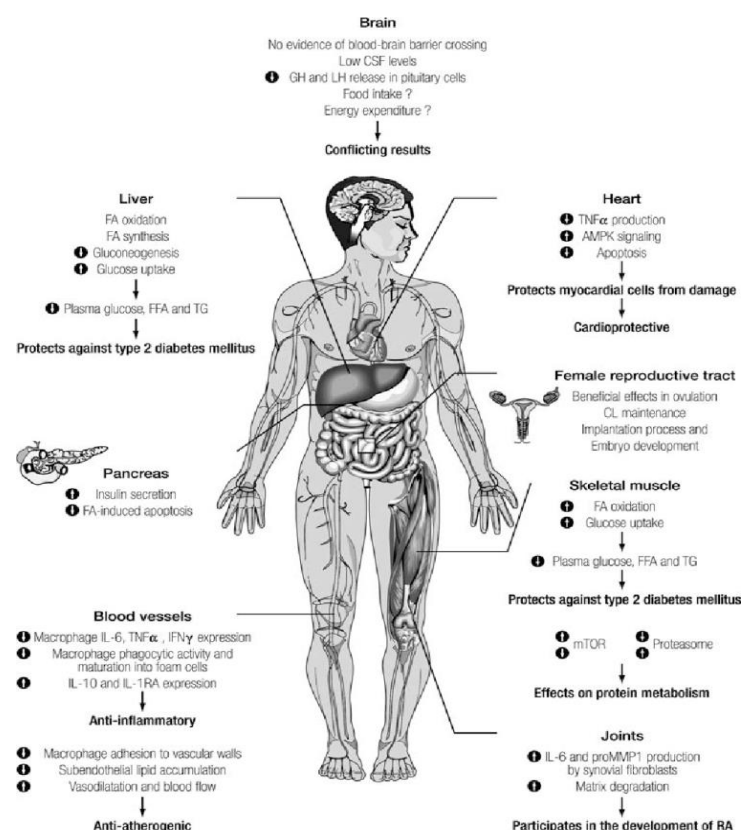


Figure 1.17:
Adiponectin actions;
Adopted from Brochu-
Gaudreau (2)

1.6 Body composition during early stages of life

Exposures during pregnancy and early postnatal life can affect growth and determine metabolic milestones in future life. The majority of studies use birthweight in order to quantify this effect. As already described, birthweight is a rough estimate of neonatal wellbeing and can only partly reflect events during intrauterine life. On the other hand, assessing body composition provides information on the natural course of chronic diseases. Precise data on fat and fat free mass allows a better understanding of the mechanisms linking neonatal malnourishment or obesity to future adverse metabolic profile. Nutritional guidelines during pregnancy and postpartum and treatment interventions to prevent obesity can be created based on body composition information.

Hydrometry, skinfold thickness, DXA, MRI and ADP are the commonest techniques for assessing body composition, using two, three or four compartment models. The two compartment model divides body to fat and fat free mass, the three compartment model divides FFM to water and fat free dry mass whereas the four (multicompartment model) further categorizes FFM to water, protein and minerals (Figure 1.18). Results derived from multicompartment models provide more detailed and precise information on body composition. They require multiple measurements using various constituents, therefore

have higher cost and longer duration. In clinical settings, measuring body composition in neonatal population is challenging. Parents prefer non interventional and time consuming techniques and neonates need to be settled to complete the assessment. In this regard, multicompartment models are not practical and methods (MRI, DXA, and ADP) using two or three compartment models are preferred. Age and gender specific constants derived from multicompartment models can be utilized by modern techniques to convert body constituents into FFM.

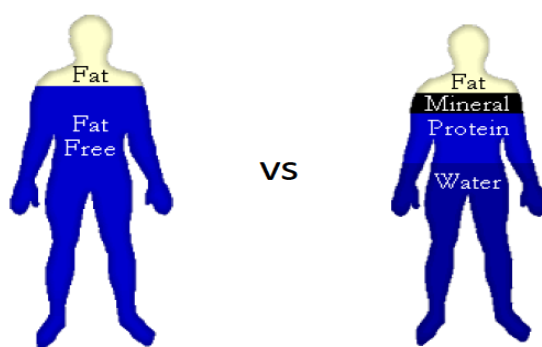


Figure 1.18: Two and four compartment models

1.6.1 Infant body composition reference data

Up to date, there is no consensus on the optimal body composition at birth (1). Age and gender specific growth charts, standardized to feeding mode and based on body composition do not exist. Growth charts proposed by various researchers are based on different techniques and cannot be used interchangeably. Each technique is based on theoretical assumptions, thus data obtained from different methods cannot be combined to create universal standards (393). So far, WHO has not defined normal growth based on body composition and existing charts are created on weight, length and BMI figures (16). The committee, after studying 8,500 children from different ethnic backgrounds, suggested that all humans should have an “optimal growth pattern”, pointing towards universal growth charts. They concluded that differences in growth are related to living conditions, therefore efforts should be made to minimize environmental effect on growth (394). On the other hand, mixed ethnicity studies have revealed that altered phenotype is not only a result of ecologic exposure but also of transgenerational effect (395). Already, in Asian adult population the threshold of BMI linked to metabolic diseases is lower compared to other populations (396). This adverse phenotype is also present at birth (“thin fat baby”) with Indian babies having more fat for a given weight when compared to white Europeans (229). The above imply that population specific data may be required

incorporating longitudinal data on cardiovascular outcomes. Tracing body composition from birth to adulthood would allow a better understanding of the pathophysiology of chronic diseases. The acquired knowledge will eventually lead to improved clinical practices, starting from early stages of life, aiming to prevent development of future obesity.

Fomon et al (397) in 1982 was the first to provide body composition reference for children up to 10 years of age. The author described his results as “preliminary and crude”, as his work was based on reports from multiple other studies and many assumptions. Butte et al (398) in 2000, after studying 76 newborns of different ethnic background produced similar body composition data up to 2 years of age (Table 1.10). Using multicompartment models author studied body composition in terms of FM, water, protein, glycogen, bone mineral content and non osseous minerals. Measurements included total body water (deuterium dilution), total body potassium (energy emission from ^{40}K) and DXA. Up to date, her work is considered as pioneer in the study of body composition and newer techniques, using two compartment models, are based on equations derived from this dataset.

Age (mo)	FFM (kg)	FM (kg)	%FM
Boys			
0.5	3.35 ± 0.40	0.44 ± 0.32	11.4 ± 8.0
3	4.37 ± 0.43	1.91 ± 0.39	30.2 ± 4.0
6	5.63 ± 0.60	2.32 ± 0.50	29.1 ± 4.7
9	6.71 ± 0.67	2.34 ± 0.64	25.7 ± 5.2
12	7.40 ± 0.72	2.56 ± 0.59	25.6 ± 4.0
18	8.55 ± 0.76	2.83 ± 0.94	24.5 ± 6.0
24	9.13 ± 1.06	3.10 ± 0.56	25.4 ± 4.7
Girls			
0.5	3.12 ± 0.45	0.52 ± 0.34	14.2 ± 9.0
3	4.11 ± 0.48	1.90 ± 0.41	31.5 ± 5.6
6	5.21 ± 0.57	2.44 ± 0.40	32.0 ± 4.4
9	6.12 ± 0.66	2.47 ± 0.46	28.8 ± 5.0
12	6.88 ± 0.68	2.62 ± 0.50	27.6 ± 4.3
18	7.99 ± 0.80	2.87 ± 0.63	26.3 ± 4.4
24	8.99 ± 1.10	3.05 ± 0.46	25.4 ± 3.9

Table 1.10: Body composition reference for children up to 3 years of age; Adopted from Butte et al (398)

1.6.2 Body composition data using ADP

Many researchers, studying various populations and using different techniques have tried to create reference data (399). As already described, results cannot be used interchangeably, therefore for the purpose of this thesis emphasis will be given to the studies using ADP as direct method of assessing body composition. Eriksson et al (400) and Carberry et al (401), studying Swedish and Australian cohorts respectively, presented data on body composition from birth to four months of age. Inclusion of both breast and formula fed neonates and mothers with raised BMI (in the Swedish cohort) were considered significant limitations of their work. Trying to address these limitations, Fields et al (402) studied 72 exclusively breastfed infants of mothers with BMI below 25kg/m² up to six months of age. Application of strict feeding criteria has led to great loss to follow up at six months, potentially biasing the results (Table 1.11).

	<i>n</i>	HC (cm)	Length (cm)	Body mass (kg)	%FM	FM (kg)	FFM (kg)
<i>Females</i>							
Birth	15	33.81 ± 1.08*	48.62 ± 1.63	3.03 ± 0.32	13.19 ± 2.27	0.40 ± 0.09	2.63 ± 0.26
1 Week	58	34.91 ± 0.97	50.13 ± 2.52	3.32 ± 0.35	12.47 ± 3.28	0.42 ± 0.14	2.90 ± 0.25
2 Weeks	66	35.27 ± 3.18	51.30 ± 2.08	3.61 ± 0.41	14.07 ± 4.15	0.52 ± 0.19	3.09 ± 0.28
1 Month	63	36.54 ± 4.20	53.49 ± 1.83	4.21 ± 0.44	19.38 ± 3.96	0.83 ± 0.23	3.38 ± 0.28
2 Months	60	38.77 ± 1.04	56.90 ± 2.29	5.12 ± 0.51	24.03 ± 3.67	1.24 ± 0.27	3.88 ± 0.32
3 Months	55	40.08 ± 1.02	59.57 ± 2.24	5.78 ± 0.58	26.56 ± 4.21	1.55 ± 0.34	4.23 ± 0.38
4 Months	49	40.91 ± 1.14	61.53 ± 2.65	6.29 ± 0.70	26.99 ± 4.62	1.73 ± 0.43	4.57 ± 0.39
5 Months	40	41.83 ± 1.03	63.23 ± 2.62	6.64 ± 0.66	27.72 ± 5.00	1.86 ± 0.45	4.78 ± 0.39
6 Months	35	42.65 ± 1.01	64.81 ± 2.37	7.13 ± 0.73	28.28 ± 4.95	2.03 ± 0.47	5.10 ± 0.47
<i>Males</i>							
Birth	20	33.80 ± 4.79	50.53 ± 1.82	3.41 ± 0.40	10.66 ± 2.84	0.37 ± 0.12	3.05 ± 0.34
1 Week	54	35.02 ± 3.20	51.22 ± 2.15	3.44 ± 0.37	11.14 ± 3.05	0.39 ± 0.13	3.05 ± 0.29
2 Weeks	71	36.40 ± 1.19	52.34 ± 2.22	3.76 ± 0.45	12.67 ± 3.90	0.49 ± 0.18	3.28 ± 0.34
1 Month	72	37.83 ± 1.11	54.60 ± 2.07	4.50 ± 0.50	18.27 ± 4.09	0.83 ± 0.24	3.67 ± 0.33
2 Months	68	39.71 ± 1.12	58.11 ± 2.29	5.56 ± 0.61	22.70 ± 4.29	1.28 ± 0.34	4.28 ± 0.38
3 Months	60	41.07 ± 1.18	61.19 ± 2.39	6.26 ± 0.70	24.65 ± 4.21	1.56 ± 0.40	4.70 ± 0.40
4 Months	54	41.96 ± 1.11	63.47 ± 2.42	6.81 ± 0.74	25.29 ± 4.29	1.74 ± 0.43	5.07 ± 0.46
5 Months	44	42.71 ± 1.43	65.33 ± 2.29	7.24 ± 0.74	26.15 ± 4.14	1.91 ± 0.45	5.33 ± 0.43
6 Months	30	43.15 ± 1.55	66.64 ± 2.74	7.40 ± 0.97	25.94 ± 4.51	1.95 ± 0.54	5.45 ± 0.55

%FM, percentage of fat mass; FFM, fat-free mass; FM, fat mass; HC, head circumference.

*Mean ± s.d.

Table 1.11: Body composition data derived from ADP in US population. Of great interest are the values at one and five months of age Adopted from Fields et al (402)

The current observational study (thesis) involves neonates born in UK, therefore population specific reference range are of great importance. Hawkes et al (403) in 2011, studying 743 Irish babies with similar characteristics to English population, triggered body composition charts at birth based on gender and gestational age. Results of his study also confirmed that maternal BMI and gestational age are independent predictors of %FM at birth. In one of the biggest cohorts, a subpopulation of the INTERGROWTH study (n=1,019) was assessed by ADP during the first 3 days of life (3). Babies born at

Oxford site were divided according to gestational age, gender and metabolic risk allowing for the creation of body composition centiles (Table 1.12, Figure 1.19). Postnatal body composition standards for UK population do not exist at present.

In summary, the above-mentioned studies utilizing ADP to assess body composition have provided similar growth patterns with small differences in measured FM and FFM. The mean combined %BF for both sexes at birth is 10-12% (SD: 4%). Newborns have a 5-10% weight loss during the first week which is driven by a reduction in FFM due to loss of water. After this period and up to 1 year of life FFM increases progressively. A rapid increase in %FM is observed during the first 6 weeks of life. Females have increased FM and reduced FFM across all age groups.

Table 1.12: Body composition data of newborns enrolled in the INTERGROWTH 21st study; Adopted by Villar et al (3); Data represents mean (SD) values

	Boys (n = 501)	Girls (n = 518)	Total (n = 1,019)	Low-risk newborns ^a (n = 247)
Age at examination, h	20.8 (16.3)	19.3 (15.2)	20.0 (15.8)	17.8 (12.9)
Gestational age at delivery, weeks	39.4 (1.6)	39.4 (1.5)	39.4 (1.6)	40.0 (1.3)
Birth weight, g	3,349 (549)	3,129 (514)	3,237 (542)	3,372 (453)
Birth weight z-score ^b	0.1 (1.0)	-0.1 (1.1)	0.0 (1.1)	0.1 (0.9)
Birth length, cm	49.3 (2.3)	48.2 (2.2)	48.8 (2.3)	49.6 (2.0)
Birth length z-score ^b	-0.1 (1.1)	-0.3 (1.1)	-0.2 (1.1)	0.1 (1.0)
Head circumference, cm	34.5 (1.4)	33.6 (1.4)	34.0 (1.4)	34.3 (1.2)
Head circumference z-score ^b	0.3 (1.0)	0.1 (1.0)	0.2 (1.1)	0.3 (0.9)
Weight to length ratio, kg/m	6.7 (0.9)	6.4 (0.8)	6.5 (0.9)	6.7 (0.7)
BMI, kg/m ²	13.5 (1.3)	13.2 (1.4)	13.3 (1.4)	13.4 (1.2)
Ponderal index, kg/m ³	27.3 (2.3)	27.2 (2.4)	27.3 (2.4)	27.1 (2.3)
Fat mass, g	332 (172)	343 (167)	337 (170)	355 (165)
Percentage body fat, %	9.6 (4.0)	10.7 (4.0)	10.2 (4.0)	10.3 (3.9)
Fat-free mass, g	2,965 (422)	2,739 (390)	2,850 (421)	2,968 (336)

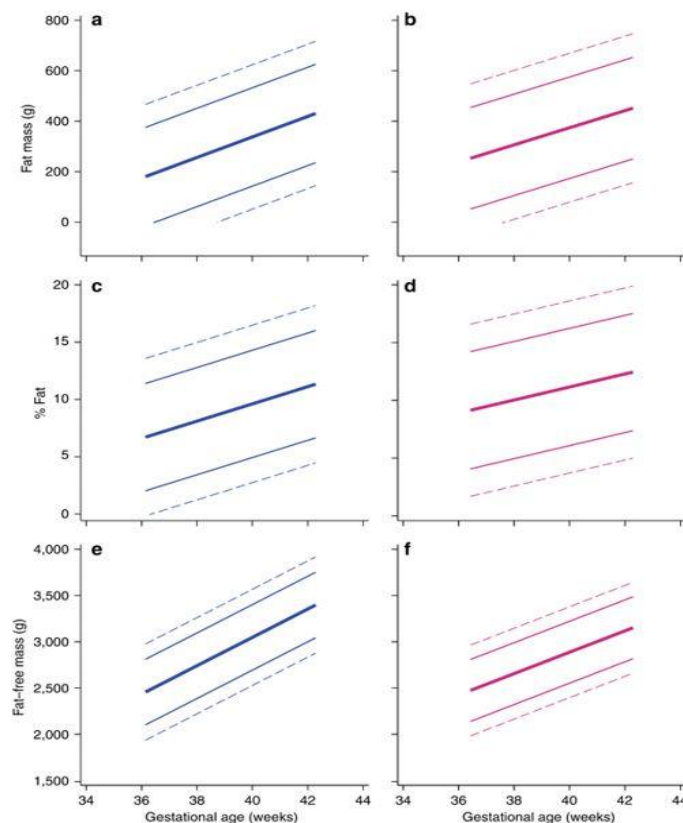


Figure 1.19: Centiles for body composition according to sex and gestational age of newborns enrolled in the INTERGROWTH 21st study; Adopted by Villar et al (14)

Blue: boys; Pink: girls

Lines represent 3rd, 10th, 50th, 90th, 97th centiles

1.6.3 Body composition measurement methods during infancy

1.6.3.1 Anthropometry

Anthropometric measurements include weight, length, regional body circumferences (arm, chest, abdominal) and skinfold thickness. Estimated body composition is derived by combining measured variables with population specific equations. These equations are based on studies using direct methods of assessing adiposity (404, 405).

Circumferences provide information on body size, proportions and fat distribution. Head circumference is used to predict adiposity rebound and abdominal circumference to assess central adiposity (406). Small head circumference at birth is related to early adiposity rebound, a predictor of obesity later in life. It has been hypothesized that fetal growth restraint and small head (brain) size at birth trigger a compensatory postnatal “catch up growth” leading to rapid utilization of fat stores and early adiposity rebound (407). In adults, abdominal circumference (circumference directly above the superior border of iliac crest) is a better marker of visceral fat than BMI (408) and is linked to diabetes, cardiovascular disease and mortality risk (409). However, studies have

revealed that use of circumferences during early stages of life has low sensitivity. Rolfe et al compared accuracy of ultrasound and abdominal circumference in predicting visceral fat in infancy against MRI derived reference data. Results revealed that waist circumference had a poor correlation with actual visceral fat, with ultrasound derived results being closely related to the MRI report (410).

Skinfold thickness is measured using Holtain calipers in pediatric population. Nine different anatomical sites have been described with subscapular and triceps skinfolds being the most commonly used. The tester needs to pinch the skin creating a double layer which includes skin and underlying adipose tissue without muscle. Skinfold is measured by applying the calipers 1 cm below the pinch. Measurement should be repeated twice. Mean of the two measurements should be recorded as the results of the test. Lower angle of the scapula is used to assess subscapular skinfolds (SS) and the midpoint between acromial and radial for the triceps skinfolds (TR; Figure 1.20). Sum of triceps and sub scapular thickness is considered a marker of total adiposity, whereas the ratio of sub scapular to triceps skinfold thickness is used to estimate central adiposity (411).

Anthropometric measurements are inexpensive, easy to obtain and not interventional. On the other hand, body composition derived from equations using skinfolds have been shown to have low correlation with MRI and hydrometry measurements in infants (412). In order to achieve low intra- and inter-observer variability testers need to undergo training and newborns need to be calm and cooperative allowing for precise readings to be obtained (413).



Figure 1.20: Triceps and subscapular skinfold thickness assessment

1.6.3.2 Hydrometry

Isotope dilution method (hydrometry) is used to assess Total Body water (TBW). Deuterium (D_2O or 2H_2O) is the commonest tracer used. Based on the dilution principle ($C_1V_1=C_2V_2$; C_1 : starting concentration, V_1 : starting volume, C_2 : final concentration, V_2 : final volume) solvent (TBW) can be estimated by knowing the volume and the concentration of the isotope (414). Enrichment of the body water pool with deuterium allows the isotope dilution space to be estimated (Figure 1.21). Baseline levels are measured by collecting neonatal saliva or urine and the same sample is collected again three hours after the isotope administration. Concentration of the tracer is measured by spectrometry and TBW values are derived. Applying age and gender specific values of the FFM hydration leads to FFM calculation (FM is anhydrous). The method is based on the assumptions that tracer is evenly distributed in all water compartments and it is not metabolized after administration.

Hydrometry is one of the most accurate and precise methods with a coefficient of variation (CV) of 1-2% (415, 416). Limitations of the method include: i) Duration of the test (4-5 hours) ii) Infants are not allowed to be fed during the test as it affects the reliability of the results iii) Due to spillage by drooling the exact amount of tracer ingested may be difficult to be assessed. The above factors have limited the use of hydrometry during early stages of life.

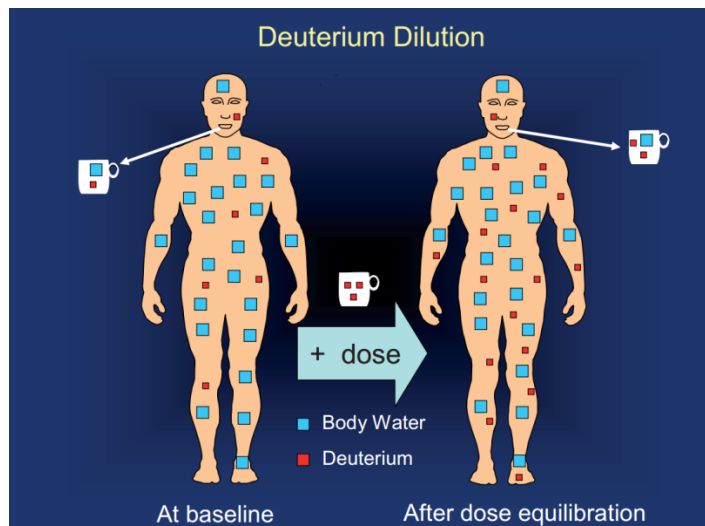


Figure 1.21: Deuterium dilution. Enrichment of the body water pool with deuterium allows the isotope dilution space to be estimated; Source: International Atomic Energy Agency (2010)

1.6.3.3 Hydrodensitometry (underwater weighing)

Archimedes, was the first to introduce the principles of hydrodensitometry, stating that “the object’s loss of weight in water equals the volume of water it displaces”. Individuals are fully submerged after a deep exhalation to minimize thoracic volume and the water displaced is measured. Knowledge of the water density allows the calculation of the volume of the body. Due to discomfort, this method is not used nowadays to assess neonatal body composition.

1.6.3.4 Multicompartment model

Assessment of body composition using multicompartment models is considered the “gold standard technique”. Body is divided into FM, TBW, protein, glycogen, bone mineral content and non osseous minerals. Each of these compartments is measured individually without the use of any assumptions. TBW is derived from deuterium dilution method, bone mineral content from DXA, nitrogen and protein from total body potassium (measured by energy emission from ^{40}K) (417). Duration of the test and exposure to small amounts of radiation (DXA) has limited the clinical use. Multicompartment models are used for the creation of reference data and the validation of new techniques (398).

1.6.3.5 Dual energy X-ray absorptiometry (DXA)

DXA scan is based on the fact that different tissues absorb different amount of radiation due to variable density and chemical composition. Two X-ray beams pass through the body, with the subject lying flat and the attenuation of radiation is measured. Body structures are classified as fat, bone and muscle (3 compartment model) (418). One of the main strengths of the technique is the provision of both whole body and regional estimates (Figure 1.22).

Validation of DXA in adults is well described but data in population <2 years of age is scarce due to radiation exposure. Radiation differs by manufacturer and a mean exposure from an infant scan is 1-5 milliSieverts, which equals the estimated effective dose of 5-25 chest x-rays (radiation dose for an infant chest x-ray is 0.02 milliSieverts) (417). Piglet carcasses have been used to validate the technique in pediatric population providing CV values for FM between 2.8 and 12.2% (419, 420). Subject movement and differences in hardware and software algorithms between manufacturers may further affect the reliability of the results (421). Overall, DXA is a very promising method in

assessing body composition providing total and regional estimates. Factors such as lack of validation studies in infants, radiation exposure, costly maintenance and radiological certification of users limit its use.

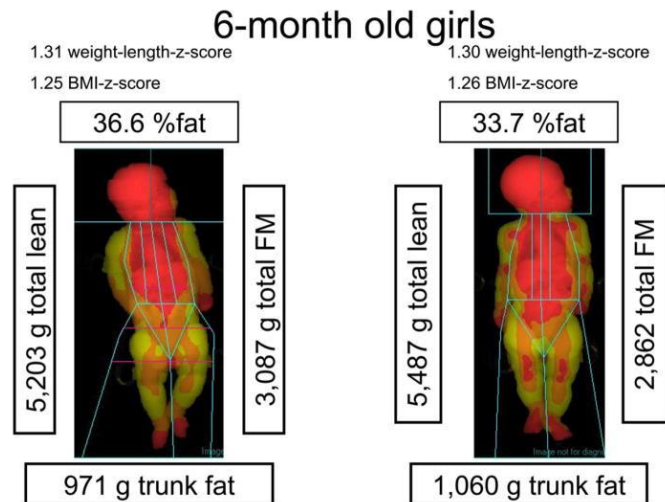


Figure 1.22: Infant body composition using DXA. Babies with similar weight have different percentage of fat mass; Adopted by Demerath et al (417)

1.6.3.6 Magnetic Resonance Imaging (MRI)

Hydrogen protons are present in all human tissues. Application of pulsed radio frequency causes the protons to absorb energy which is released back once the field is switched off. The detected signals are used by the receiver to create images (422). MRI provides data on both total and regional body composition volumes. The test does not involve radiation exposure allowing for repeatability in infants. Studies have revealed a high reproducibility (CV=2.6-3.4%) and accuracy for subcutaneous fat (CV=1.6%) with a lower precision for visceral fat (CV=8.7%) (423, 424).

MRI application in infants has significant limitations. Subjects need to remain still as movement artifacts affect the quality of the images. Test is technically difficult as infants need to be scanned while sleeping and wearing earplugs. Pulse oximeter needs to be used during every test, monitoring baby for crying and discomfort. Limited access to MRI scans, high maintenance cost and need for technically trained staff reduce the practicality of this method (417).

1.6.3.7 Air displacement Plethysmography (ADP)

ADP is the method of using body density to derive body composition. Based on the 2 compartment model, body is divided into FM and FFM. Body density is calculated by dividing mass by volume (Density=Mass/Volume). Mass is acquired by weighing the subject with an accurate weighing scale whereas volume is measured in air using Boyle's and Poisson's laws.

Boyle's equation describes the behavior of air when temperature remains stable (Figure 1.23) and is: $P_1V_1=P_2V_2$, where P_1 , initial pressure; V_1 , initial volume; P_2 , final pressure and V_2 , final volume.

When air changes temperature in response to volume changes (adiabatic conditions) the equation is converted to: $P_1/P_2=(V_2/V_1)^\gamma$, where γ for air is 1.4 (Poisson's law). Adiabatic conditions mean that there is no external factor contributing to the change in temperature except the heat produced by the "work". For small volume changes P_2 is always 40% larger than P_2 in isothermic conditions, or in other words it is easier to compress air under isothermic conditions.

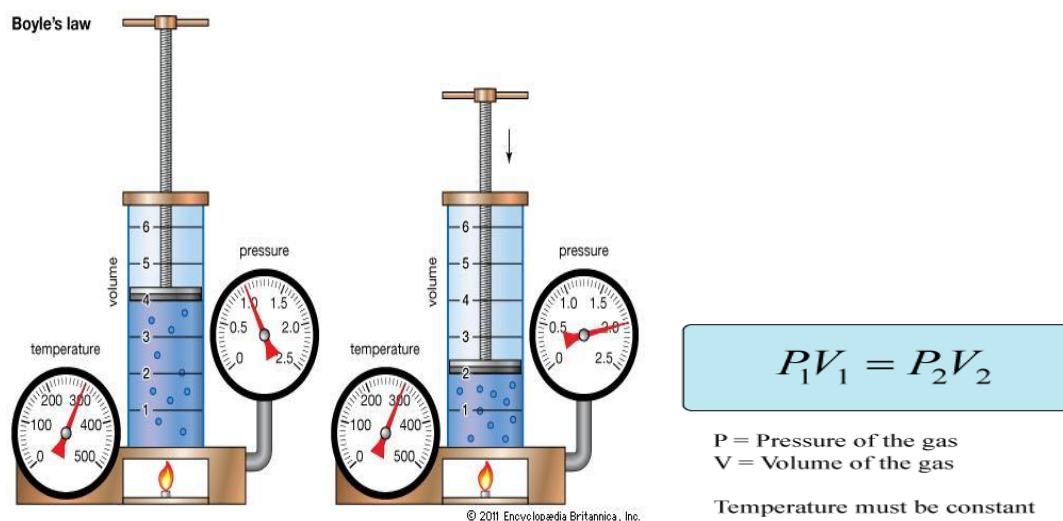


Figure 1.23: Inverse relationship between volume and pressure of gas under isothermic conditions

“PEAPOD Cosmed Infant Composition system” is the only commercially available ADP technique for infants, first introduced in 2003 (Figure 1.24). It is licensed to measure infants up to 8kgr in weight, which equals approximately 6 months of age. It is safe, fast and reliable technique in assessing body composition in this very challenging age group.

Operators do not require any specific training and system can accommodate infant's behavior (crying, urination, defecation) without altering the accuracy of the results (see below). One of the main disadvantages of the technique is that the child/adult version (BodPod) is licensed for children above 2 years of age, leaving a gap between 6 and 24 months of age where body composition cannot be assessed by ADP when longitudinal data is required.

The PEAPOD components are placed on a movable cart. Test chamber, electronic scale, monitor are on the top while reference chamber, volume calibration container, air circulation, heating system, computer and printer are mounted inside the cart (Figure 1.25). Electronic weighing scale has a capacity of 12kgr. A diaphragm separates the test and reference chamber whereas a pneumatic valve is between test and calibration chamber. Both test and reference chamber have a volume of 37 L each. Insertion of a subject in the test chamber causes the diaphragm to oscillate resulting in volume perturbations in the two chambers which have equal magnitude but opposite sign. Calibration chamber is smaller with a 5L volume. During calibration pneumatic valves open allowing communication between test and calibration chambers.



Figure 1.24: PEAPOD (Infant Body Composition assessment)

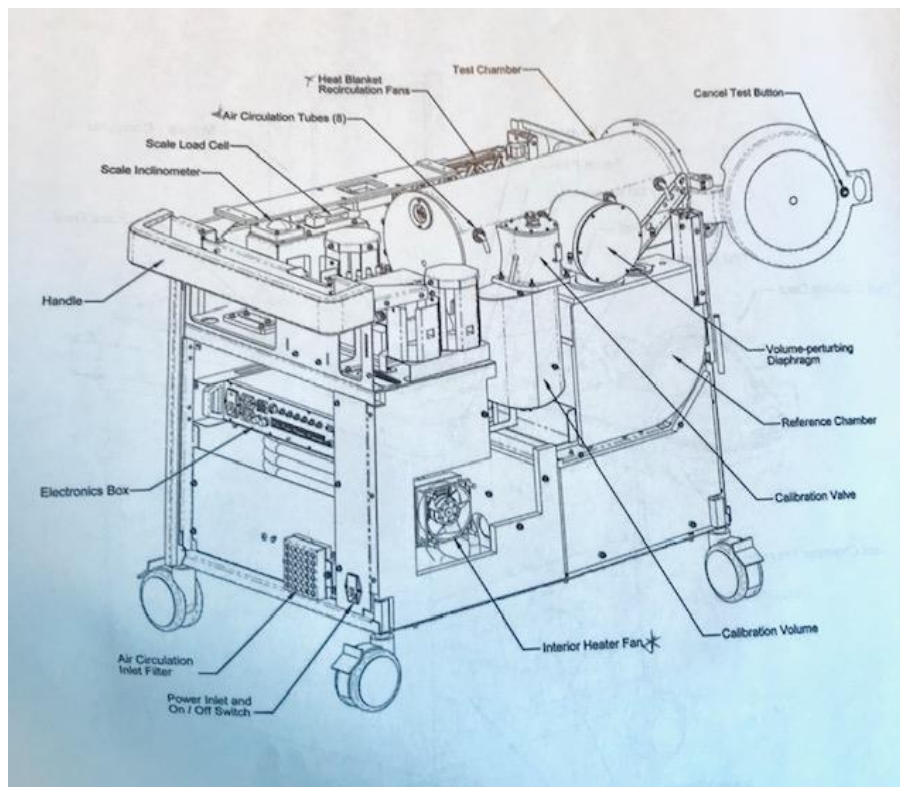


Figure 1.25: PEAPOD parts. Schematic demonstration of the interior parts of the PEAPOD

1.6.3.7.1 Operating principles

Using Boyle's and Poisson's equations an unknown volume (V_{test}) can be calculated by the ratio of pressures in the test (P_{test}) and reference chamber (P_{ref}). The oscillating diaphragm reflects the change in pressure induced by volume change. Magnitude of pressure change is inversely related to the volume in the chamber resulting in the following equation:

$$V_{\text{test}} = (P_{\text{ref}}/P_{\text{test}}) \cdot V_{\text{ref}}$$

Air close to subject's body, hair and within lungs behaves isothermally. PEAPOD assumes that all air in the test chamber is under adiabatic conditions resulting in a 40% overestimation of air behaving isothermally. System automatically corrects for the behavior close to subject's surface (Surface Area Artifact, SAA) and within lungs (Thoracic Gas Volume, TGV). SAA is dependent of length and weight and is calculated based on Boyd equation (425) :

SAA = [178.27*Length_(cm)^{0.5}* Weight_(kg)^{0.4838}]*k, where K is a constant based on the effect of surface area on volume.

Lung volume is overestimated by 40% leading to equal underestimation of body volume. PEAPOD uses prediction algorithms to predict TGV based on pulmonary research literature and adjusted for weight, length and gender (426). TGV is calculated as the sum of Functional Residual Capacity (FRC) and half of the Tidal Volume (TD; Figure 1.26).

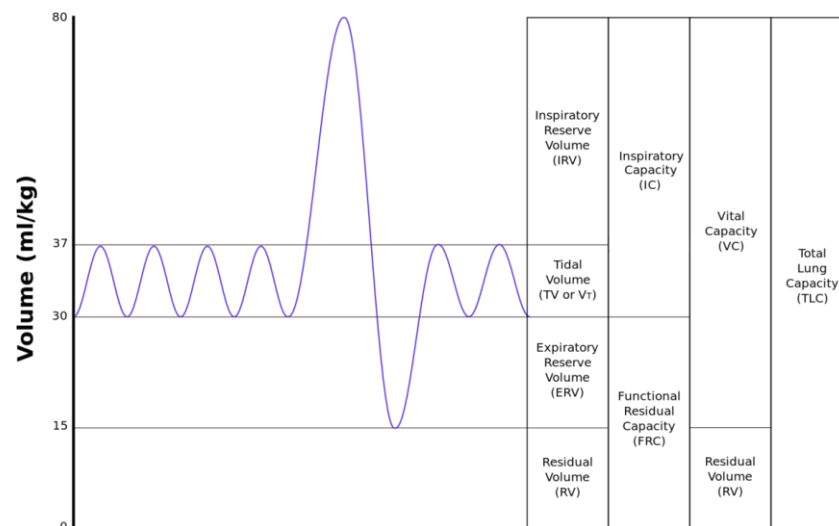


Figure 1.26: Lung volumes and capacities

Taking into considerations the above adjustments total body volume is calculated as:

$$V_{\text{body final}} = V_{\text{raw body volume}} - \text{SAA} + 40\% \text{ TGV}$$

1.6.3.7.2 Calculating FM and FFM

Body density is derived from mass and volume. Applying the 2-compartment model and considering that density is a function of the proportions and densities of its components lead to the following equations:

$1/D_B = FM/D_{FM} + FFM/D_{FFM}$ where D_B , D_{FM} and D_{FFM} are densities of body, FM and FFM respectively. Replacing FFM with 1-FM leads to:

$$\% \text{Fat} = [(D_{FM}D_{FFM}/ D_B(D_{FFM}-D_{FM}) - (D_{FM}/D_{FFM}-D_{FM})] * 100\%.$$

Using data from Fommon et al (397) and Butte et al (398) models FFM is predicted based on gender and age whereas FM density remains constant at 0.9007kgr/L. Once %Fat is calculated, FM, FFM and %FFM are easily derived from:

$$\%FFM = 100 - \%Fat$$

$$FM = (\%Fat)(M_B) / 100\%$$

$$FFM = \text{Total Body Mass} - FM$$

1.6.3.7.3 Test procedure

To maintain the integrity of the PEAPOD system quality control needs to be performed daily. Users should leave at least two hours between switching on the application and testing allowing PEAPOD to warm up and reach desirable temperature in the test chamber (31°C). Weighing scale should be checked against reference 5kg phantom on daily basis. Every two weeks or if PEAPOD cart is moved weighing scale requires calibration to rule out the unlikely scenario of altered gravity at different geographical locations. At the beginning of each test day or if there is more than 6-8 hours interval between tests, volume calibration needs to be performed to assess PEAPOD and environmental stability. The 5Lt reference phantom is inserted in the test chamber and six consecutive volume measurements are obtained. If standard deviation between tests is less than 0.003Lt calibration is considered successful. Room temperature needs to be maintained between 20-28°C and ambient change in temperature between and during tests should be $\leq 0.5^\circ\text{C}$. PCO_2 levels should be checked before test and kept $\leq 0.45\%$. Users are allowed to proceed to subject testing when all quality control criteria are met.

With electromagnetic door closed and test chamber empty automatic volume calibration is performed at the beginning of each test. Different pressures are applied to the diaphragm with the pneumatic valve open or closed. The communication between test and calibration chamber allows the system to perform analysis of the inverse correlation between different pressures and volumes.

At the same time, infant is undressed and prepared for mass measurement. For sanitary reasons insulation material (paper, sheet) is placed and left on the scale before calibration, during mass measurement and post test calibration. Infants are placed supine and in case of urination or defecation test is repeated. Data including gestational age, date of birth, gender, length and unique study number is entered in the screen. By completion of data entry and mass measurement, volume calibration is usually finished and the door opens automatically. Plastic tray is pulled out and infant is placed in the supine position. In case of excessive hair, few drops of oil are used to flatten the hair. Tray is pushed back and the door is closed. During volume measurement pressure changes are measured from the diaphragm's oscillation. Assessment lasts two minutes

and door opens spontaneously. Results of the test are displayed on the screen (Figures 1.27 and 1.28).

Infant's behavior should be monitored throughout the test. In case of excessive crying and increasing PCO₂ levels, fitted sensor in the test chamber automatically opens the door and aborts the test. If during the test user or parents feel uncomfortable or are concerned for the infant's behavior, test can be aborted by using one of the two safety buttons which allow the door to open (Figure 1.29). Test is repeated with parental consent once infant is settled. After each test scale and test chambers are cleaned using alcohol free disinfectant wipes or cleaning solution recommended by the manufacturer.



Figure 1.27: Steps in assessing infant body composition using PEAPOD. Infant is weighed in the electronic scale and then placed in the plastic tray for volume assessment

Figure 1.28: Detailed display of body composition results



Figure 1.29: PEAPOD safety buttons (indicated by red arrows)

1.6.3.7.4 ADP validation

Pediatric ADP has been validated against reference methods in 9 studies (Appendix 1) (427). For most researchers the “4 compartment” approach (4-C) is the gold standard method in assessing body composition. Ellis et al (428) examined 49 infants using ADP and compared the results with reference values from 4-C model, obtained after TBW, total potassium and DXA measurements. A subgroup of infants (n=31) had a repeat ADP assessment within 15 minutes to check reliability of the method. The two methods were significantly correlated (R^2 : 0.73, $p < 0.001$) and a good agreement was observed with 95% limits of agreement at -6.8 and 8.1 %FM. Mean difference of the measured %FM was 0.6 ± 3.7 ($p = 0.62$). Results also revealed reproducibility of the ADP tests, reporting a within subject CV of 7.9% for %BF and mean difference between first and second measurement of 0.4% ($p > 0.05$).

Detailed results of ADP reliability and accuracy were presented by Ma et al in 2004, (429) using deuterium solution as the reference method. Accuracy was assessed in 53 infants of various body weight (2.7-7.4 kgr) and reliability in 36 infants after three tests in two consecutive days (day 1 test 1, day 2 test 1, day 2 test 2). Each ADP test included three volume measurements and the effect of infant's behavior (cry, sleep, active, urinate or defecate) on calculated volume was assessed. In terms of reliability, results revealed mean %FM difference of -0.5 ± 1.21 and 0.16 ± 1.44 ($p > 0.05$) for between and within days measurements respectively. Within days %FM CV was 5.1 ± 0.65 and between days CV was 4.94 ± 0.62 . Infants behavior had no significant effect on calculated %FM ($p = 0.69$). Mean %FM obtained by ADP did not differ significantly from that obtained from deuterium dilution (mean difference 0.07%, $p = 0.89$). Bland Altman analysis revealed narrow agreement limits between the two techniques (95% agreement limits: -6.84, 6.71 %FM).

Evaluation of ADP has also been assessed in bovine tissues against chemical analysis technique (430). The study included bovine phantoms of different weight and muscle to fat mass ratio in order to represent compositions similar to both term and preterm babies. Mean %FM difference between the 2 methods was 0.04 ($p=0.91$) with narrow agreement limits at -1.22 and 1.13%. Repeat ADP measurements on the phantoms revealed consistent CV for %FM above 10% (CV <5%) whereas precision was lower for lower fat mass range (CV: 18.14%). Similar results were presented by Frondas-Chauty et al using live piglets (431).

Up to date studies confirm that ADP is an accurate and reliable method of assessing body composition during infancy. Poor precision noted in animal models with low range %FM has not been observed in inanimate phantoms (432). Furthermore, given that the majority of human infants have >10% FM makes ADP suitable for measurement of body composition in clinical practice (186). In very preterm infants, characterized by significantly low FM, repeated ADP measurements (2-6 tests) allows the extraction of very precise results (430, 433). ADP has been validated against reference techniques reporting narrower agreement limits than other methods (DXA) (434). Of great significance is the fact that results were consistent across a wide range of weight and FM values.

In summary, numerous methods are available in assessing infant body composition. Clinicians should take into account the cost of the measurements, practicality, compliance of the studied population and the assumptions of the underlying principles of each technique (Table 1.13). ADP can be reliably used for the assessment of body composition in longitudinal studies including infants, a group where compliance is difficult to be achieved.

Methods	Accuracy	Practicality	Ethical
Skin-fold thickness	Improper site identification and fold grasping	✓	✓
Hydrometry	✓	Impossible due to compliance	Impossible due to age
Hydrodensitometry	✓	Impossible due to compliance	Impossible due to age
DXA	More studies required	Subject needs to remain stable	Exposure to radiation
MRI	✓	Subject needs to remain stable; High cost	✓
ADP	✓	Gap between 6 months and 2 years	✓

Table 1.13: Body composition techniques in infancy. One of the main disadvantages of the ADP technique is the lack of equipment to measure body composition in early childhood

1.7 Study methods and main objectives

Intrauterine exposures can set offspring metabolic trajectories with long lasting effects. It is therefore of vital importance to identify the missing link between adverse maternal characteristics and offspring adiposity. In order to understand the origin of metabolic diseases detailed data on the body composition during early stages of life is required. Studying the prognostic value of fetal and neonatal biomarkers in predicting future obesity will allow early identification of high risk groups and prompt implementation of prevention strategies.

In order to answer the above questions we performed a systematic review (chapter 2) looking at the associations between early life biomarkers (cord blood leptin and adiponectin) and adiposity up to 5 years of age. We also performed a longitudinal, observational study looking at the effect of clinical and biochemical maternal characteristics on infant body composition (chapters 3, 4 and 5). Finally, using data from

our observational study we assessed the precision and reliability of traditional anthropometric equations in predicting infant adiposity, using an objective measurement of infant body composition (PEAPOD) as reference (chapter 6).

1.7.1 Hypothesis

Adverse maternal metabolic profile, such as obesity, gestational weight gain, ethnicity and GDM, is linked to increased risk of offspring adiposity, a strong predictor of adult metabolic dysfunction. We hypothesize that this altered body composition is present at birth and during the early stages of life in offspring born to high risk mothers, suggesting early life programming of obesity and metabolic disease.

1.7.2 Research Questions

- Is maternal adverse metabolic profile linked to altered body composition at birth and up to 5 months of age? (Chapters 3, 4 and 5)
- Is there any association between cord leptin and adiponectin with body composition, assessed by air displacement plethysmography, at birth and up to 5 months of age? (Chapter 5)

1.7.3 Outcomes and criteria

- Assess differences in body composition (fat and fat free mass) in newborns of mothers with (case) and without (control) GDM (Chapter 3; in a nested case-control study)
- Assess whether the common high-risk factors for GDM (BMI, age, ethnicity) could independently predict offspring adiposity, in the absence of GDM (Chapter 4)
- Study if different patterns of neonatal body composition are related to mid-pregnancy maternal markers (insulin, glucose, leptin, adiponectin) and early life biomarkers (cord blood leptin and adiponectin) (Chapter 5)
- Compare if data obtained with traditional methods (anthropometry and skin-fold thickness) are similar to these obtained from ADP (Chapter 6)

1.7.4 Inclusion criteria

- All pregnant women between 18-45 years who delivered at George Eliot Hospital
- All pregnant women between 18-45 years who had their antenatal care at George Eliot Hospital and had a home birth

1.7.5 Exclusion criteria

Congenital abnormalities, identified either during anomaly scan or immediately post delivery

1.8 Summary

In recent years, childhood obesity has reached epidemic proportions. Children with obesity will remain obese as adolescents and adults, with high cardiometabolic risk. Given the significant co-morbidities linked to obesity, the rising rate represents one of the biggest social and financial burden of our days. Treating obesity has been proven to be challenging therefore identifying strategies for its prevention is of crucial relevance. Genetics are responsible for a small proportion of BMI and the rapidly increasing rates of obesity cannot be explained by genetic predisposition. The sedentary lifestyle and the plethora of food high in fat and sugars, characteristics of our modern world, are undoubtedly contributing to the obesity epidemic. There is increasing evidence though, that events occurring during in utero and early postnatal life may imprint on offspring leading to future adverse metabolic profile and non-communicable diseases. Adverse exposures, such as maternal obesity, undernutrition and GDM, during the plastic developmental period may program the offspring to develop obesity and metabolic diseases in future life. The mechanisms underpinning these associations, although not fully understood, appear to be related to multitude of factors including altered epigenome and impaired hormonal levels affecting the development of hypothalamus with long-lasting adverse effects. Animal studies have provided some insight on the pathophysiology but the exact mechanisms related to developmental programming are yet to be identified. Defining critical developmental periods and susceptibility to diseases based on early life events will allow the development of prevention strategies and interventions with practical effect. Studying the association between adverse maternal environment and neonatal body composition is a promising field which will shed more light to the exact mechanisms of developmental origins of obesity. Weight, weight gain and BMI are indirect measures of obesity/adiposity, therefore defining exact levels of FM and FFM during early life will allow interventions targeting body composition during the period of developmental plasticity to avoid future adverse metabolic phenotype. Finally, as objective methods of assessing body composition are not yet widely available, discovering early life biomarkers to predict future adiposity will allow the early identification of high risk groups and the prompt initiation of intervention strategies.

In this regard, the main aim of the current thesis is to assess how adverse maternal environment affects offspring's body composition during the first months of life and to study whether early life biomarkers, specifically, cord blood leptin and adiponectin are linked to future adiposity.

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CHAPTER 2

Role of cord blood leptin, adiponectin and infant body composition: Systematic Review and Meta-analysis

2.1 Introduction - Adipocytokines in pregnancy

Insulin and IGF-1 are the most well described growth factors during pregnancy (1). As the role of intrauterine exposure in the development of future metabolic diseases is established, finding a link between fetal markers and risk of adverse metabolic profile would enable us to understand the mechanisms underlying this association. Leptin and adiponectin may play an important role in fetal growth either having a direct effect or acting as mediators and sensitizers to insulin and IGF-1.

2.1.1 Leptin in pregnancy and early post natal life

During pregnancy leptin is produced by maternal adipose tissue, placenta and fetal tissues. Maternal leptin increases as pregnancy advances with highest levels noted during third trimester. Levels are higher when compared to non pregnant subjects, adjusted for total FM (2). The above observation can be explained by increased fat accumulation during the pregnancy and predominantly during the last trimester. Researchers also believe that the higher leptin levels for a given FM could also be attributed to placental leptin contribution. Furthermore, the increased amount of placental hormones, HCG, insulin and oestradiol noted in pregnancy, factors known to promote leptin expression, contribute to leptin's higher levels (3). Leptin resistance could explain the unique biological behavior of leptin noted in pregnancy. Hyperleptinaemia is not accompanied with loss of appetite and increased energy expenditure, as it would be expected (4). More likely, leptin plays a protective role for fetal well being, promoting metabolic efficiency required before delivery. In detail, during periods of maternal malnutrition leading to low maternal leptin, placental leptin affects maternal metabolism, ensuring that all reserves are utilized by the growing fetus and not used to restore maternal supplies. The role of leptin in mobilizing energy stores for offspring's best interest is further supported by the fact that levels are higher in BF mothers compared to those non lactating post partum (5).

Data has been controversial regarding maternal leptin and neonatal anthropometry. The majority of studies found no correlation between maternal leptin and birthweight (6-8) whereas a recent study from Brunner et al (9) showed an inverse correlation with birth weight and FM. Similar results were presented in two large cohorts by Retnakaran et al (10) and Verhaeghe et al (11). The discrepancy may be attributed to different timing of maternal serum leptin sampling. On the other hand, pregnancies complicated by

maternal obesity are characterized by increased cord leptin levels, a correlation which is more likely driven by fetal adipose tissue accumulation (12, 13).

Leptin mRNA and leptin receptors have been detected in human placenta, using PCR (14). 98% of leptin drains in the maternal circulation whereas only small amounts are transferred to the fetus (15). The above observation paired with the fact that maternal leptin cannot cross the placenta due to high molecular weight, support the independent fetal leptin production. Multiple reports present that umbilical artery has higher levels of leptin compared to umbilical vein (7, 16), strengthening the data on the “two compartment model” between maternal and fetal leptin production.

Fetal leptin has been detected as early as 18 weeks of gestation, with levels rising as pregnancy progresses, in concordance with fetal fat accumulation (17). Leptin expression in the fetus varies depending on environmental/external conditions. In ovine models, fetal hypoxia, hypercortisolaemia, hyperinsulinaemia and hypothyroidism promote leptin production whereas fetal hypoglycaemia and hypoinsulinaemia have the opposite results (18). High levels of gene expression for leptin and its receptors have been found in many fetal tissues, including fetal osteoblasts and chondrocytes, lungs, CNS, intestine, kidneys and hair follicles (19). The early detection of leptin, the presence in multiple tissues during fetal life and the higher levels seen in newborns than children after adjusting for adiposity suggest a role as growth factor (20). Multiple studies have confirmed the involvement of leptin in the development of many fetal tissues (Figure 2.1). Javaid et al (21) showed that cord leptin levels can predict bone mass at birth. His results were further supported by Fu et al (22) who proved that leptin regulates bone formation by affecting osteoblast proliferation. Leptin is also involved in fetal lung maturation. Both fetal lung fibroblasts and epithelial cells express leptin and its receptors and are part of a paracrine loop. Epithelial cells secrete parathyroid hormone related protein which promotes leptin expression from fibroblasts, which in return results into increased expression of surfactant from the epithelial cells (23). Islam et al (24) proved the presence of leptin receptors on rat islet cells and its role on fetal pancreatic cell proliferation. At the same time, the widespread presence of leptin receptors throughout the digestive tract shown by Aparicio et al (25) strengthens the evidence on the role of leptin as a growth factor of fetal gastrointestinal system.

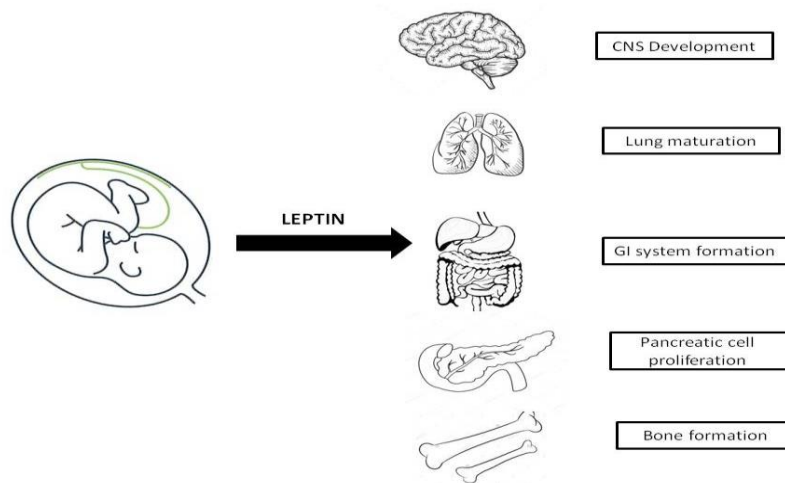


Figure 2.1: Role of leptin during fetal life; CNS: central nervous system, GI: gastrointestinal

Of great importance is the role of leptin in the development and migration of neuronal cells. In humans hypothalamic circuits which determine feeding behavior and satiety in later life develop during late antenatal and early postnatal life (26). Hormones and environmental factors in the early post partum can affect the ARC and mitochondria function, leading to changes which can persist throughout life. Several studies, predominantly in animal models have shown that the early post partum is a period of plasticity, in which interventions can imprint genotype for generations. IUGR piglets are born with low leptin levels resulting in reduced ObR expression in the hypothalamus and altered adipocyte morphology with increased lipid storage capacity. The above effect is reversed after leptin treatment (27). There is more evidence to support that leptin treatment in the early stages of life can affect neurotrophoblasts and regulate feeding and weight gain, thus preventing future obesity. Ob/Ob mice (28) as well as undernourished rats (29) have greater weight gain and measures of adiposity as adults when compared to controls. These associations are reversed after early leptin treatment, which has shown to normalize feeding behaviors and metabolic markers in future life. It is crucial to understand though that it is the early post natal life which can provide a window for interventions to modulate intrauterine programming. Bouret et al (28) showed that leptin treatment in Ob/Ob mice in adulthood has no effect on the neuronal connections in the hypothalamus, which is in contrast with early life treatment which restores structure (30).

Leptin levels drop during the first five days of life. Cold, fasting and to a lesser extent lack of placental contribution lead to this observation. During suckling period (day 5-6) there is a surge in leptin levels, not related to FM, responsible for the neurotrophoblasts'

maturation. The timing and the duration of this peak seem to set long term feeding behaviors and metabolic trajectories. In particular, lambs from mothers with obesity have a premature peak of leptin followed by reduced amplitude and prolonged duration when compared to normal fed offsprings. The early post natal surge is mainly driven by raised cortisol levels (Figure 2.2) (31). On the other hand, undernourished offspring demonstrate premature leptin surge which results in greater weight gain in the following weeks (Figure 2.3) (32).

The application of the knowledge acquired from animal models is of great importance in cases of SGA babies. LGA babies have higher leptin levels when compared to AGA at birth and cord leptin is lower in SGA when compared to AGA babies (33). These associations are driven by total FM. It would be interesting to adjust leptin levels for total FM, as it would provide evidence regarding early leptin resistance in babies born small for their gestational age. Preterm or SGA babies have low leptin levels at birth but by 1 year of age their levels are higher than normal (34). The low leptin levels at birth regulate feeding behaviors by modulating hypothalamic connections and lead to rapid “catch up” growth and weight gain in infancy. As a result they are at higher risk of developing diabetes and obesity in future life (35). The above data once again suggest that intrauterine and early post natal life are crucial periods for metabolic programming and development of future diseases.

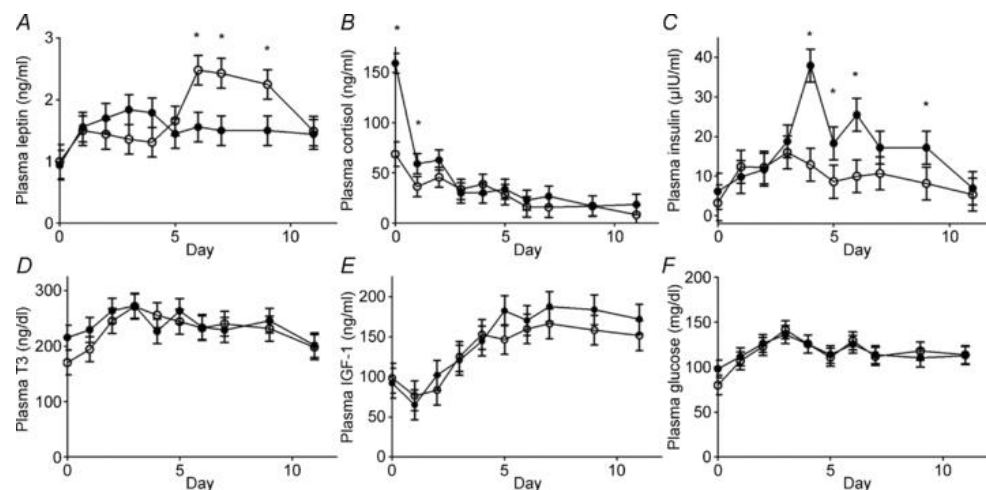


Figure 2.2: Circulating plasma levels in lambs from mothers with obesity (filled circles) and controls (open circles). The early leptin surge is driven by higher cortisol levels Adopted from Long et al (31)

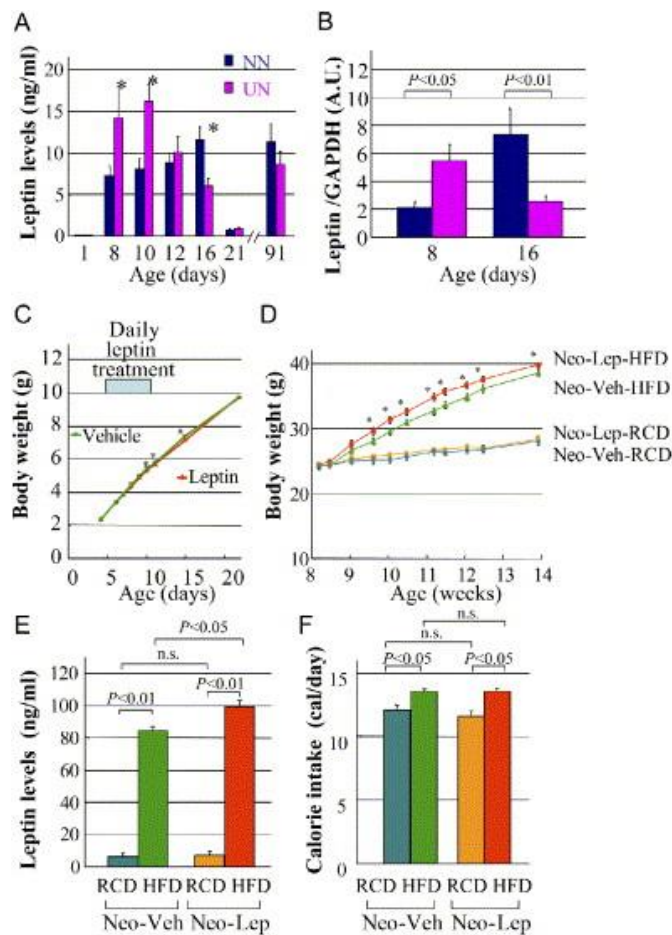


Figure 2.3: In utero malnourishment leads to premature leptin surge and higher weight gain (A, B). Marked body weight changes are present in NN offspring fed HFD postnatally (C, D). Leptin treated offspring have higher leptin levels but similar caloric intake, suggesting that increased energy expenditure mediates leptin's role in early life. Dark and light orange lines (C, D) and columns (E, F) indicate neonatal leptin treatment; NN: normal nourished, UN: undernourished, HFD: high fat diet, RCD: regular chow diet. Adopted from Yura et al (32)

Data has shown no correlation between maternal and cord blood leptin (9, 36), confirming the autonomous origin of fetal leptin. On the other hand maternal BMI and weight gain during pregnancy positively predict leptin at birth (12, 37, 38). As more nutrients cross the placenta, fetal glucose and insulin levels rise, leading to adipose tissue accumulation and leptin production. Kadakia et al (39) focusing on the molecular origins of hyperleptinaemia, showed that increased pre-pregnancy BMI leads to reduced leptin gene methylation. DNA methylation of a gene promoter leads to decreased gene expression. Advancing gestational age, linked to increasing fetal FM deposition, is positively related to cord leptin. Many studies have tried to investigate the association between cord leptin and anthropometry at birth. Karakosta et al (40) published a systematic review including cross-sectional, observational and case control studies of uncomplicated full term pregnancies. Studying different ethnic populations proved that

cord leptin positively predicts birth weight ($r=0.47$, CI: 0.43, 0.52) and has a weaker correlation with length ($r=0.29$ CI: 0.23, 0.34) and ponderal index ($r=0.36$ CI: 0.31, 0.41). In order for leptin to be established as a marker of future obesity, longitudinal studies comparing cord samples with anthropometric measurements at later stages of life are required. As methods of assessing body composition are not widely available, researchers used weight/weight gain or BMI as a marker to investigate this association. The ALSPAC Study team in 1999 (35) was one of the first to report an adverse relation between cord plasma leptin and weight gain to 2 years of life. Similar results have been demonstrated recently by Brunner et al (9) studying a cohort of 188 newborns to 2 years of age. As the data was getting stronger over the years, researchers started to look into high risk groups and special populations. Fonseca et al (41) reviewed 96 premature babies and found that cord leptin could negatively predict the rate of change in BMI and length up to 2 years. In a big cohort of 578 Greek babies, Karakosta et al (12) found that cord leptin inversely predicted weight at 4 years of age in AGA but this association followed a positive trend in SGA. The above observation suggested earlier onset of leptin resistance in the SGA group.

As described before, a short plasticity window for interventions in the early post natal life is well documented in animal models. In order to identify this optimal therapeutic period and the onset of leptin resistance studies looked into leptin levels and offspring's anthropometry at different time points. Mantzoros et al (42), in one of the biggest cohorts so far ($n=588$), confirmed that cord leptin is inversely related to BMI at 3 years of age but also found that serum leptin at the age of 3 positively correlates with adiposity at this age. Boeke et al (43) followed participants from the above cohort (Project Viva) up to 7 years of age. They proved that serum leptin at 3 years is positively related with adiposity and BMI at 7 years of age. The latest two reports suggest that reduced leptin sensitivity or even leptin resistance starts within the first 3 years of life. As childhood obesity is difficult to treat once it is established, interventional programs to prevent the development should take place at the early stages of life. Early life biomarkers, such as leptin, which could be obtained from a single blood test and are related to future obesity, will enable the early identification of high risk individuals and will allow targeted approach to prevent the development of metabolic diseases in the future.

2.1.2 Adiponectin in pregnancy and early post natal life

The anti-diabetic and anti-atherogenic role of adiponectin has been the main focus of research during the last decades. Recently the potential role of adiponectin in intrauterine

growth has drawn the attention of many researchers. In particular, the different metabolic profile and action during fetal life compared to adults has led to the hypothesis that adiponectin may act as a growth factor and not only as a marker of adiposity.

During pregnancy, adiponectin is isolated from maternal and fetal circulation, whereas placental production remains controversial. Early stages of pregnancy are characterized by high maternal adiponectin levels, resulting in accumulation of fuels in maternal stores. (44) During the second half, maternal adipose tissue increases and adiponectin levels drop, leading to fuel mobilization (lipids, glucose) to supply the fetus in order to achieve normal intrauterine development and growth (45). Due to its HMW maternal adiponectin does not cross the placenta (44) and is not related with cord blood levels (46, 47). Results regarding maternal levels and birth weight remain contradictory. Weyermann et al (47) studying a big cohort of newborns (n=766) found no correlation between maternal adiponectin and weight at birth whereas other studies, including healthy mothers with various BMI and pregnancies complicated with GDM, revealed a negative correlation (48-50).

Maternal adiponectin has been associated with diseases during pregnancy. Low levels outside pregnancy are related to increased GDM risk in subsequent pregnancies (51) whereas low levels during early pregnancy positively predict the development of hyperglycaemia at later stages (52). Mothers with GDM are found to have lower levels compared to normal pregnancies (53, 54). Iliodromiti et al (55) suggested that first trimester adiponectin could be used as a predictive tool of GDM development. Performing a systematic review showed that the Area Under the Curve (AUC) of adiponectin for GDM is 0.78 (95%CI 0.74, 0.81). There is increasing interest on the role of adiponectin in the development of pre-eclampsia and gestational hypertension. Although the exact mechanism is not yet understood, low first trimester levels increase the risk of hypertension as pregnancy progresses (56). Pre-eclampsia is characterized by lower adiponectin levels at early pregnancy but higher during delivery when matched with normotensive mothers (57).

Similarly to leptin, it was initially believed that placenta produces adiponectin. (58, 59) However recent studies, using more sensitive techniques, have shown it is unlikely that the placenta expresses and produces adiponectin (60, 61). This discrepancy between studies can be explained by the fact that studies suggesting placenta as a source of adiponectin used cell lines rather than fetal tissues. It is believed that the results are due to contamination of the cell lines from the maternal and fetal bovine serum. Sivan et al (62) in a cohort of 51 neonates showed that cord blood adiponectin levels were similar to

those four days post partum, confirming that cord adiponectin is derived from fetal and not from maternal or placental production.

It is widely accepted that placenta expresses AdipoR1 and to a lesser extent AdipoR2 receptors (63). Fetal growth depends on the delivery of nutrients across the placenta. The materno-placental transport is determined by maternal nutritional status, utero-placental blood flow and the ability of placenta to express trophoblast nutrient transporters. Placenta is not only a communication canal, the presence of adiponectin and other adipocytokines receptors, suggests a role as a nutrient sensor, receiving signals from both maternal and fetal environment in order to balance fetal demand with maternal fuel supply. Fetal adiponectin levels reveal the nutritional status of the fetus and through placental signaling maternal energy stores are mobilized (64). On the other hand, maternal adiponectin is shown to inhibit placental proliferation in cell lines and attenuate the trophoblast transporter expression (65). The above observation could potentially explain the inverse correlation between maternal adiponectin and size at birth, however, as mentioned before, results are not consistent. In detail, conditions like GDM and maternal obesity, characterized by low maternal adiponectin levels, lead to hypertrophic placenta allowing more fetal nutritional supply, thus enhancing fetal growth and size.

Cord adiponectin levels increase by 20-fold from midgestation to term and levels are positively related to gestational age (66). In 2005, Corbetta et al (67) using PCR and Western blotting in cadaveric fetuses between 14-36 weeks gestation showed that adiponectin is expressed in fetal tissues other than brown and white adipose tissue, suggesting a role as growth factor. Skin, skeletal muscle, small intestine, arterial walls and amniotic membranes were found to express adiponectin mRNA whereas placenta, consistent with previous results, was found not to produce adiponectin. It still remains unknown if adiponectin has a direct effect on fetal tissue or its role is mediated by other hormones (Figure 2.4). During antenatal life insulin is the main growth factor and the tool to utilize maternal glucose, therefore adiponectin may act as an insulin sensitizer. In favor of this hypothesis, Tsai et al (68) showed that LGA babies have higher adiponectin but similar insulin levels when compared to AGA. Insulin action mediated by higher levels in the LGA group was enough to result in increased weight and adiposity. In summary, the lack of correlation between maternal and cord levels, the stable adiponectin levels during early postnatal life and the presence of adiponectin mRNA in various fetal tissues suggest an important role during intrauterine development.

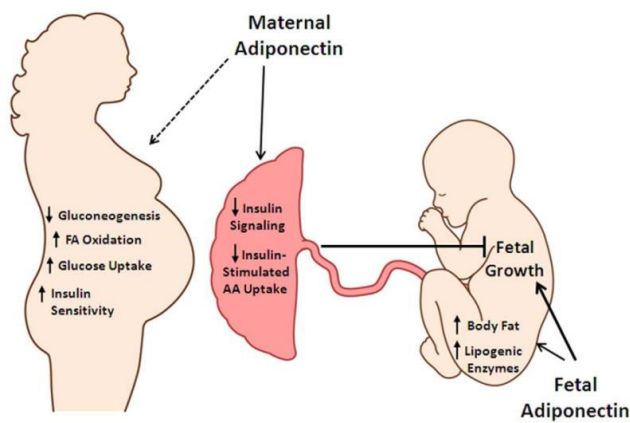


Figure 2.4: Role of adiponectin during pregnancy; Adopted from Aye et al (64)

Of great interest, adiponectin presents a different metabolic profile in fetal and neonatal life compared to this seen in adults. The inverse correlation between adipose tissue and adiponectin seen in adult population is not present during the early stages of life. The correlation between adiponectin, weight and FM during fetal and neonatal period remains controversial (discussed below). Furthermore, cord blood levels are significantly higher compared to adults, after adjusting for total FM (69, 70). There are many possible explanations for the shift from “positive to negative” correlation between adiponectin and FM, occurring around school entry age (71). The multiple sites, other than adipose tissue, of adiponectin expression seen in fetuses is a main contributing factor. Similar to anorexic adult patients, the low percentage of FM seen during the early stages of life leads to an impaired negative feedback to adiponectin production (72). Furthermore, as humans get older the structure and distribution of adipose tissue changes. Brown adipose tissue is reduced (73) and the subcutaneous to visceral fat ratio follows the same trend (74), leading to reduced adiponectin production. Fat cells become hypertrophic and the production of pro-inflammatory cytokines inhibits adiponectin expression (75). Finally, adipocytes become less “efficient” as mitochondria reduce in numbers or lose their function (76).

Unlike leptin, the association of adiponectin with anthropometric measurements at birth and its role as a predictive marker of future adiposity have not been extensively investigated. Data on the correlation between birth weight and cord adiponectin levels remain controversial. Lindsay et al in 2003 (77) and Martinez et al (78) two years later showed no correlation between weight at birth and cord adiponectin. Similar results were presented by Teague et al in 2015 (79), after studying a cohort of 125 neonates. On the other hand, Tsai et al (68) in a big cohort of full term neonates (n=225) showed that cord adiponectin levels positively predict birth weight. In line with his results, two more studies

(46) revealed a positive association in Asiatic and Caucasian populations. In a longitudinal study of 588 offsprings, Mantzoros et al (42) showed that cord adiponectin is inversely related to weight gain during the first 6 months of life but fails to predict BMI at 3 years of age. It is difficult to explain what causes this discrepancy between various studies. Variability of the commercial kits used to measure adiponectin levels and the different adiponectin multiforms (total vs. high molecular weight vs. globular) examined in each study are potential contributors. Insulin is the main growth factor antenatally. Adiponectin is a known insulin sensitizer, enhancing the role of insulin during intrauterine life. The change of its metabolic profile after birth, with growth hormone taking over the role of the main growth factor, could potentially explain its inability to predict BMI in early childhood. Despite the convincing evidence of adiponectin's role in intrauterine development, studies report mixed results. We therefore did a systematic review and metaanalysis of all the available studies that reported cord blood adipocytokines (specifically Leptin and Adiponectin) and their association with body composition of the children at birth and early childhood.

2.2 Cord blood adipocytokines and body composition up to 5 years of age: a systematic review and meta-analysis

2.2.1 Background

Adipose tissue in infancy is essential in ensuring adequate energy supply to the brain in a period of nutritional disruption and acts as thermo-insulator (white adipose tissue) and thermo-regulator (brown adipose tissue) (80). While it has a protective role against infections, intensive care unit admissions and mortality (81, 82), the recent evidences show that the strongest predictors of adult obesity are large for gestational age (LGA) and obesity in preschool children. However, the optimal range of adiposity in children which defines “beneficial versus metabolically harmful” levels of FM is yet to be identified (83). To address this, researchers proposed body composition growth charts as opposed to birth weight charts to improve the predictive ability of future obesity (84). Others used cord blood adipocytokines such as leptin and adiponectin as objective markers to predict future obesity compared to adiposity measurements, which are subjective and prone to measurement errors.

As previously described, up to date, researchers have tried to find the correlation between cord blood leptin and adiponectin with birthweight and BMI in childhood. There

is convincing evidence that cord blood leptin positively predicts birthweight and is negatively related to BMI and weight gain up to 3 years of age. On the other hand, there is paucity of data regarding adiponectin's role and the findings remain inconsistent. The disadvantage of this literature is that weight or BMI is not a reliable marker of adiposity. In order to explain the mechanisms behind intrauterine and early life programming of future diseases, detailed body composition is required (FM and FFM). Published studies examining the association between cord blood adipocytokines and adiposity at birth show mixed results. Most were cross-sectional and in small cohorts (9, 68, 85-96). Thusfar there have only been 3 moderately sized longitudinal (42, 43, 97) examining the link between cord blood levels and childhood adiposity up to 7 years of age. It is difficult to draw firm conclusions from these studies due to different ethnic groups sampled and different methods in assessing body composition. The purpose of this systematic review is to summarize these studies to improve the power and to shed light on the role of cord blood leptin and adiponectin on adiposity at birth and in early childhood.

2.2.2 Hypothesis

Leptin and adiponectin are key hormones for the energy homeostasis in adults. Their role during fetal period is less clear, with evidence suggesting that apart from mirroring adipose tissue, they also act as growth factors imprinting offspring's future phenotype. We hypothesize that cord blood levels reflect fat mass at birth. We also hypothesize that cord leptin levels are related to adiposity in early childhood through adverse programming of hypothalamic appetite centers and increased cord adiponectin levels are associated with increased future adiposity.

2.2.3 Research Questions

- Is there any association between cord blood leptin and adiponectin with body composition at birth?
- Is there any association between cord blood leptin and adiponectin with body composition in children up to 5 years of age?

2.2.4 Methods

2.2.4.1 Search strategy and study selection

Systematic reviews collect and critically analyze primary research studies in order to provide answers to research questions. The increasing number of publications in health research combined with the limited time clinicians can spend on reading available literature, makes systematic reviews a very useful tool. They are the cornerstone of evidence-based medicine, as many of the clinical guidelines are based on reliable systematic reviews. Roberts et al (98) though, has shown that there is an increasing number of systematic reviews which are biased or even out of date when published, highlighting the need for universally agreed standards and guidelines.

Observational, cross-sectional and longitudinal studies were examined. Studies of the offspring of healthy pregnant women and those with obesity were included. For studies involving participants with diabetes (type 1 or 2, gestational diabetes), data obtained only from control groups was used in the meta-analysis, as offspring of mothers with diabetes have higher leptin concentrations for a given FM (99, 100). Studies of full term neonates of different growth patterns (AGA, LGA, SGA) and ethnic origin were considered eligible. Studies examining neonates born preterm or/and with congenital abnormalities (chromosomal disease, respiratory distress syndrome, heart and renal disease) were excluded (Table 2.1). Randomized control trials were not included in the current systematic review. Due to the different criteria used to define SGA, LGA and obesity across various populations, we accepted the authors' definition.

Cord blood samples, measuring leptin and adiponectin, analyzed by Enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) qualified for the meta-analysis. Leptin results were reported in ng/ml and adiponectin in µg/ml.

We included studies that assessed neonatal and childhood adiposity by Air Displacement Plethysmography (ADP), Dual Energy X-ray Absorptiometry (DXA), Magnetic Resonance Imaging (MRI) and anthropometric measurements (skinfold thickness). Studies using only ponderal index as a measure of adiposity were excluded.

The review was registered on PROSPERO (CRD42017069024, Appendix 2). Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines were followed for the study (101). Since the review involved synthesis of published data NHS Research

Ethics Committee approval was not required. The individual studies included obtained approval from the respective local research ethics committee.

A literature search was performed between January 1994 (discovery of leptin gene) and November 2018. Two bibliographic databases were used to conduct the searches: Medline/Pubmed (National Library of Medicine and National Institute of Health) and EMBASE (The Excerpta Medica Database). The following keywords and medical subject headings (MeSH) were used: “leptin”, “adiponectin”, “fetal blood”, “umbilical cord blood”, “adiposity”, “obesity”, “body composition”, “fetal growth” and “anthropometry”. Search words were combined using Boolean operators (AND, OR). The search was limited further to those studies published in English and performed in humans from birth to 5 years of age. Reference lists from included studies were reviewed for further potentially eligible articles (Appendix 3).

Inclusion criteria	Exclusion
Observational, cross-sectional	Randomized control trials
Healthy term pregnancies	Preterm pregnancies
Any ethnic background	Pregnancies complicated by diabetes
Any growth pattern (AGA, SGA, LGA)	Congenital anomalies
Adiposity assessed by DXA, MRI, PEAPOD and skinfold thickness	Adiposity assessed only by ponderal index

Table 2.1: Inclusion and exclusion criteria used for the study selection

2.2.4.2 Data extraction

Level one screening of search results (title and abstracts) was performed by two independent reviewers {Christos Bagias, Nithya Sukumar (Clinical Lecturer, Warwick Medical School, Warwick)], using the inclusion and exclusion criteria provided. Level two screening of the full manuscripts was conducted by them independently (CB and NS) and any discrepancy was resolved by consulting a third one (CB’s PhD supervisors: Ponnusamy Saravanan, Oyinlola Oyebode).

For studies fulfilling the inclusion criteria, two independent review authors extracted details onto standard data extraction templates. This included information about type of study, location and time of data collection, population, sample size, type of leptin and adiponectin assays, statistical methods and technique to assess body composition. Any disagreement was resolved by discussion or by consulting a third review author.

2.2.4.3 Statistical analysis

Partial correlation and multiple regression models were used to present the results. Studies were reported narratively, when correlation coefficient (r) was unable to be extracted. Meta-analysis of correlations was performed using a random effects model. Cochran's Q test was used to assess heterogeneity and I^2 statistics to calculate the percentage of variation across studies that was due to heterogeneity rather than chance. Different methods of assessing body composition were used, therefore the test of moderators was applied to investigate the effect on heterogeneity. Forest plots were created for each outcome. The potential for publication bias was assessed using funnel plots when the requirement of ten or more studies per meta-analysis was met. Egger's test to assess funnel plot's asymmetry was applied. All analyses were conducted using STATA version 14 in collaboration with supervisor's institution (Warwick Medical School) and Dr Yonas Weldeselassie (Lead Statistician for Professor Saravanan).

2.2.4.4 Assessment of risk bias

The assessment of methodological quality of the studies was done using checklist criteria. This systematic review includes observational cohorts and cross-sectional studies, therefore the quality assessment tool adopted from the National Institutes of Health/ National Heart, Lung and Blood Institute was used (Appendix 4 and 5). After answering a series of 14 questions, quality of the studies was reported as poor, fair and good (Table 2.2).

Study	Quality		Study	Quality
Meyer, 2018 (104)	i		Simon-Muela, 2013 (94)	ii
Meyer, 2017 (105)	i		Basu, 2009 (95)	i
Schneider, 2017 (103)	i		Mantzoros, 2009 (42)	ii
Kadakia, 2016 (92)	ii		Inami, 2007 (96)	i
Karakosta, 2016 (97)	ii		Valuniene, 2007 (90)	i
Chaoimh, 2016 (91)	ii		Martinez, 2005 (78)	i
Donnelly, 2015 (85)	ii		Javaid, 2005 (87)	ii
Teague, 2015 (88)	ii		Tsai, 2004 (102)	ii
Josefson, 2014 (89)	ii		Lindsay, 2003 (77)	i
Brunner, 2014 (9)	ii		Geary, 1999 (106)	ii
Boeke, 2013 (43)	ii		Clapp, 1998 (93)	i

Table 2.2: Assessment of included studies; Quality was assessed using quality assessment tool adopted from the National Institutes of Health/ National Heart, Lung and Blood Institute and rated as 0 for poor, i for fair, ii for good

2.2.5 Results

After applying our search criteria, 164 studies were identified, which were reduced to 148 after removing duplicates. After title and abstract screening 118 articles were excluded, leaving 30 for full manuscript review. Twenty-two studies (9, 42, 43, 68, 77, 85-97, 102-106) met all the inclusion criteria. Figure 2.5 presents the selection of the studies included in the literature review.

Search words: Leptin, adiponectin, fetal blood/umbilical cord blood, adiposity/obesity, body composition, fetal growth, anthropometry

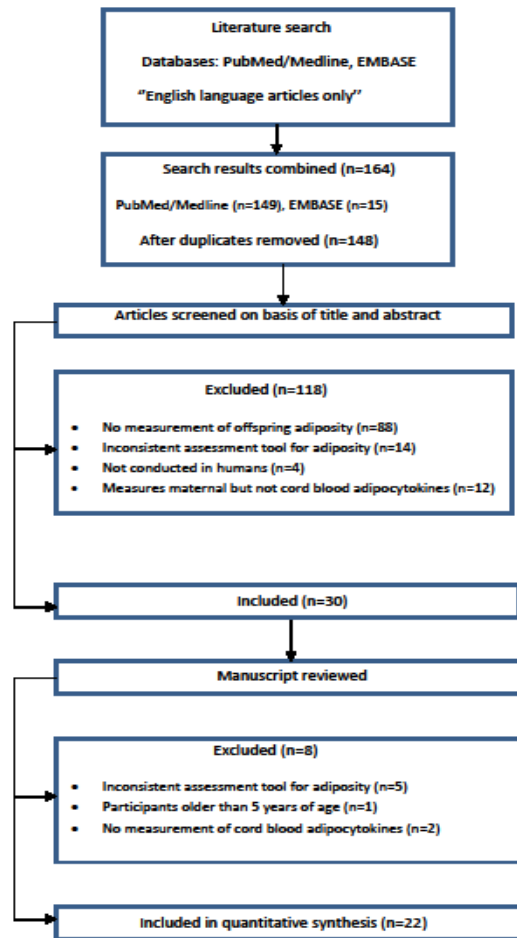


Figure 2.5: Flow diagram of the study. After screening 164 articles and removing duplicates, 22 studies were included for the final analysis.

2.2.5.1 Characteristics

Table 2.3 provides baseline characteristics of the studies included. Most of the studies examined Caucasian neonates while one study (103) examined an African American population. Three studies (39, 88, 89, 92) included neonates of more than one ethnicity. In terms of maternal characteristics and risk factors, most of the studies included mothers without diabetes or other metabolic disorders. Two studies (78, 102) reported the association between adipocytokines and neonatal adiposity based on intrauterine growth (SGA vs AGA, AGA vs LGA).

2.2.5.2 Studies reporting leptin

The correlation between leptin and adiposity was investigated in 17 studies (9, 42, 43, 85-93, 97, 102-104, 106). Eleven (9, 85-93, 102) examined the relationship at birth using either Pearson correlation or multiple regression analysis. Eight (9, 42, 43, 88, 91, 97, 103, 104) assessed the relationship between cord blood leptin and adiposity at different time points (3 weeks, 2 months, 2-5 years of age) using partial correlation or multiple regression models. Eleven studies (42, 85-87, 89, 90, 92, 93, 102, 103, 106) used radioimmunoassay (RIA) as the method of measuring cord blood leptin whereas six (9, 43, 88, 91, 97, 104) used enzyme-linked immunosorbent assay (ELISA). Body composition was assessed using DXA (n=1) (87), ADP (n=4) (9, 89, 91, 92), MRI (n=1)(104) and skinfold thickness (n=12) (9, 42, 43, 85, 86, 88, 90, 93, 97, 102, 104, 106). Total body fat was derived by measuring skinfold thickness at two [triceps(TR) and subscapular(SS)] (42, 43, 85, 93, 97), three [TR+SS+quadriceps(QD)] (90), four [TR+SS+QD+suprailiac(SI)] (9, 78, 79) or six (TR+SS+QD+SI+ biceps+gastrocnemius) sites (102, 104).

2.2.5.3 Studies reporting adiponectin

The correlation between adiponectin and adiposity at birth was examined in eight studies (86, 88, 89, 94-96, 102, 105), reporting correlation coefficient. Martinez et al (78) described no association between cord blood adiponectin and adiposity. For the purpose of statistical analysis this was considered as $r=0$. Basu et al (95) reported different correlation coefficients between male and female participants. Four studies (42, 88, 103, 104) looked at the association between cord adiponectin and body composition at different age groups (1-3 months, 3-5 years of age). Adiponectin was measured using either RIA (n=6) (42, 77, 78, 89, 102, 103) or ELISA (n=5) (88, 94-96, 105). Two studies used ADP to calculate body composition (89, 103), one MRI (105) and the remaining used skinfold thickness at different sites {2 sites (n=2) (42, 105), 4 sites (n=5) (78, 88, 94, 96), 6 sites (n=1) (102)}.

Two studies (77, 106) reporting association between cord plasma adipocytokines and isolated skinfold measurements (not derived total adiposity) were excluded from further analysis.

2.2.5.4 Cord blood leptin and neonatal adiposity at birth

All 11 studies (9, 85-93, 102), assessing 1653 pregnancies, revealed a moderate positive correlation between cord blood leptin and neonatal FM ($r = 0.487$; 95%CI: 0.444, 0.531; Figure 2.6). Heterogeneity between studies was assessed ($I^2=22.3\%$, $p=0.14$) and hence a random effects model was fitted. As different methods of assessing adiposity was used (DXA, ADP, Skinfold thickness) we included 'method' in the random effects model to examine the effect on heterogeneity. The results showed that there was no evidence for heterogeneity between the type of adiposity measures (QM test for moderators, $p=0.50$). Therefore, random effects model without the covariate 'method' was fitted.

Eight studies reported mean levels of cord blood leptin (42, 43, 88, 89, 97, 102, 104). Applying continuous random effects model revealed a pooled mean value of 9.1 ng/ml [95% CI 8.27, 9.95; $p<0.001$]. Heterogeneity was present ($I^2=85.76\%$, $p<0.001$).

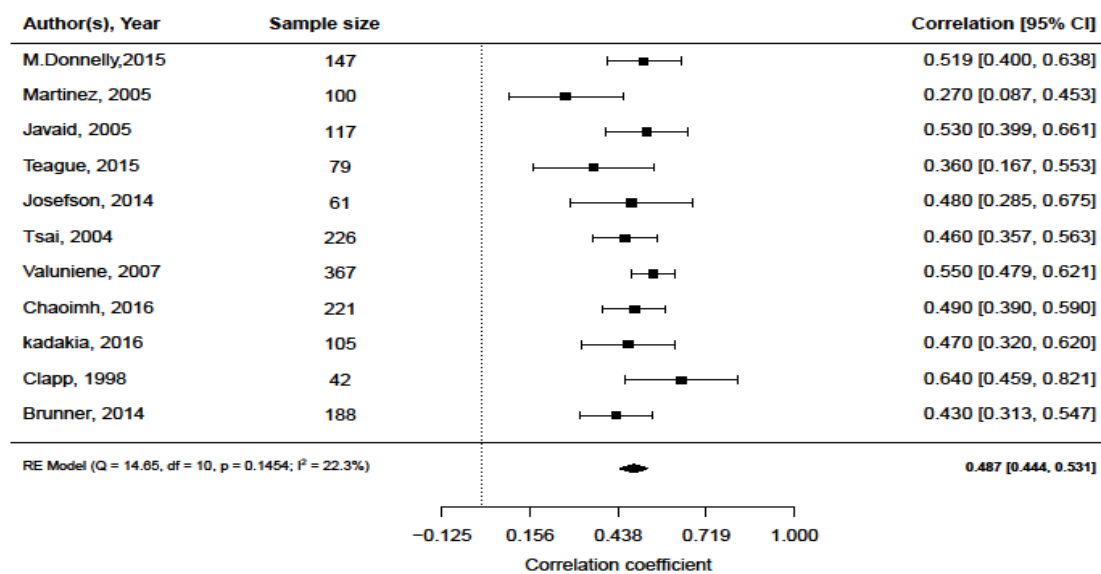


Figure 2.6: Forest plot showing the pooled correlation between cord blood leptin and fat mass at birth

Study	Country (Sample size)	Maternal age	Maternal BMI	Gestational age	Birthweight	Leptin	Adiponectin assessment	Adiposity method
Meyer, 2018 (104)	Germany (n=89)	NR	NR	NR	NR	ELISA	-	Skinfold, MRI
Meyer, 2017 (105)	Germany (n=90)	NR	NR	NR	NR	-	ELISA	Skinfold, MRI
Schneider, 2017 (103)	USA (n=36)	22.5 (3.7)	29.1 (8.2)	39 ⁺⁸ (1)	3347 (380)	RIA	RIA	ADP
Kadokia, 2016 (92)	USA (n=105)	32 (3.8)	25.0 (6.1)	39 ⁺⁵ (1.0)	3502 (515)	RIA	-	ADP
Karakosta, 2016 (97)	Greece (n=578)	29.5 (4.9)	NR	38 ⁺⁰ (1.0)	3195 (442)	ELISA	-	Skinfold
Chaoimh, 2016 (91)	Ireland (n=221)	NR	NR	40 ⁺² (39.4, 41.4)*	3510 (3290, 3760)*	ELISA	-	ADP
Donnelly, 2015 (85)	Ireland (n=147)	32.6 (4.2)	26.9 (4.7)	40 ⁺³ (1.0)	4080 (480)	RIA	-	Skinfold
Teague, 2015 (88)	USA (n=124)	24.6 (4.4)	28.2 (6.9)	39 ⁺⁶ (0.9)	3400 (400)	ELISA	ELISA	Skinfold, DXA
Josefson, 2014 (89)	USA (n=61)	NW: 32.8 (5.9) O: 30.2 (4.5)	NW: 22.0 (1.8) O: 35.5 (4.0)	NW: 39 ⁺⁸ (1.1) O: 39 ⁺⁸ (1.0)	NW: 3380 (470) O: 3600 (570)	RIA	RIA	ADP
Brunner, 2014 (9)	Germany (n=188)	NR	NR	NR	NR	ELISA	-	Skinfold
Boeke, 2013 (43)	USA (n=508)	NR	NR	NR	NR	ELISA	-	Skinfold
Simon-Muela, 2013 (94)	Spain (n=96)	M: 31.0 (4.6) F: 31.8 (5.0)	M: 25.5 (5.9) F: 24.4 (4.92)	M: 39 ⁺¹ (1.7) F: 39 ⁺⁷ (1.5)	M: 3255 (535) F: 3319 (487)	-	ELISA	Skinfold
Basu, 2009 (95)	USA (n=121)	M: 29.1 (5.6) F: 26.5 (5.7)	NR	M: 38 ⁺⁷ (0.7) F: 38 ⁺⁸ (0.6)	M: 3406 (532) F: 3199 (445)	ELISA	ELISA	Skinfold
Mantzoros, 2009 (42)	USA (n=588)	32.3 (5.1)	24.8 (5.4)	39 ⁺⁷ (1.5)	NR	RIA	RIA	Skinfold
Inami, 2007(96)	Japan (n=52)	NR	NR	NR	3054)	-	ELISA	Skinfold
Valuniene, 2007 (90)	Lithuania (n=367)	NR	NR	SGA: 38 ⁺⁸ (1.6) AGA: 39 ⁺¹ (1.8)	SGA: 2398 (374) AGA: 3507 (501)	RIA	-	Skinfold
Martinez, 2005 (78)	Mexico (n=100)	SGA: 26.5 (5.1) AGA: 25.2 (5.4)	NR	SGA: 38 ⁺⁰ (37, 40)* AGA: 39 ⁺⁰ (37, 41)*	SGA: 2305 (133) AGA: 3754 (388)	RIA	RIA	Skinfold
Javaid, 2005 (87)	England (n=117)	28 (5.6)	NR	M: 40 ⁺¹ (1.1) F: 39 ⁺⁶ (1.3)	M: 3506 (511) F: 3251 (461)	RIA	-	DXA
Tsai, 2004 (102)	Taiwan (n=226)	NR	NR	LGA: 39 ⁺⁶ (1.2) AGA: 38 ⁺⁶ (1.0)	LGA: 4070 (210) AGA: 3350 (330)	RIA	RIA	Skinfold
Lindsay, 2003 (77)	Scotland (n=73)	NR	NR	NR	NR	-	RIA	Skinfold
Geary, 1999 (106)	England (n=39)	29.7 (6.1)	24.2 (3.8)	NR	3442 (539)	RIA	-	Skinfold
Clapp, 1998 (93)	USA (n=42)	NR	NR	NR	3600 (360)	RIA	-	Skinfold

Table 2.3: Characteristics of studies included, maternal age is expressed in years, gestational age in weeks and birthweight in grams. Figures in () represent standard deviations. NR: not reported, NW: normal weight, O: obese, M: male, F: female, AGA: appropriate for gestational age, SGA: small for gestational age, LGA: large for gestational age, ADP: Air Displacement Plethysmography, DXA: Dual Energy Absorptiometry, MRI: Magnetic Resonance Imaging, RIA: radioimmunoassay, ELISA: enzyme-linked immunosorbent assay) *Figures in () express interquartile range

2.2.5.5 Cord blood leptin and adiposity up to 5 years of age

All studies reported a negative correlation between cord blood leptin and weight gain to 4 years of age (9, 42, 43, 88, 91, 97, 103, 104). Schneider et al (103) studying a small number (n=36) of African American babies found no correlation between cord leptin and adiposity at 2 weeks and 3 months of life. On the other hand, Chaoimh et al (91) (n=221) found that cord leptin and adiposity at 2 months of age are negatively related ($b = -0.021$, $p = 0.003$). Brunner et al (9) (n=118) and Boeke et al (43) (n=508) demonstrated a negative correlation between cord leptin and adiposity at 2 and 3 years of age respectively, after adjusting for maternal and offspring characteristics. Similarly, Mantzoros et al (42) (n=588) found a negative relationship with adiposity at 3 years of age ($b = -0.24$) which was not statistically significant ($p = 0.48$). However, it is likely that this study and the one by Boeke et al (43) are from the same cohort (Project Viva). Meyer et al (104) following a cohort of 89 offsprings showed that cord leptin was negatively associated with total adiposity (predicted from skinfold thickness equations) at 3 and 5 years of age, but with small effect size (3 years: $b = -0.02$, $p = 0.03$; 5 years: $b = -0.03$, $p = 0.03$). The association was not observed when adipose tissue was directly assessed by MRI at 5 years in a subgroup of 33 children (visceral adipose tissue: $b = 0.26$, $p = 0.7$; subcutaneous adipose tissue: $b = 0.13$, $p = 0.9$). Studies reviewed (43, 97) revealed a positive, but not statistically significant, association with adiposity at 4 and 7 years of age (Table 2.4).

Meta-analysis was not performed as results were adjusted for different maternal characteristics (age, gestational age, BMI, education, income, smoking, breastfeeding).

Study (Sample size)	Cord leptin (ng/ml) to	Adjusted for	Results	P value
Meyer, 2018 (104) (n=89)*	%FM at 3 and 5 years VAT(cm ³) and SAT(cm ³) at 5 years	Maternal BMI, gestational weight gain, pregnancy duration, sex, breastfeeding	B-Coef {95% CI} %FM at 3: -0.06 {-0.13, 0.01} %FM at 5: -0.09 {-0.17, 0.00} VAT: 0.26 {-1.71, 2.23} SAT: -0.13 {-9.20, 8.94}	p = 0.07 p = 0.04 p = 0.78 p = 0.97
Schneider, 2017 (103) (n=36)	FM(g) at 2 weeks and conditional change from 2 weeks to 3 months	2 weeks: gestational age, age at measurement, FFM 3 months: above+ 2 weeks measurement and time between measurements	2 weeks: r = 0.27 3 months: r = -0.19	p>0.05
Karakosta, 2016 (97) (n=578)	SSF(mm) at 4 years of age	Sex, birthweight, maternal age and education, parity, pre-pregnancy BMI and breastfeeding duration	B-Coef {95% CI} : 0.2 {-1.4, 1.7}	p >0.05
Chaoimh, 2016 (91) (n=334)	Conditional change to FMI(kg/m ²) from birth to 2 months	Maternal age-education-smoking, maternal BMI at 15weeks gestation, family income, sex, gestational age, breastfeeding	B-Coef {95% CI} : -0.021 {-0.034, -0.007}	p <0.05
Teague, 2015 (88) (n=52)	%FM at 1month	Diabetic status, sex, age in days	r = 0.19	p = 0.19
Brunner, 2014 (9) (n=90)*	FM(g) at 2 years	Maternal BMI, gestational weight gain, pregnancy duration, sex, breastfeeding	B-Coef {95% CI} : -14.86 {-29.49, -0.23}	p= 0.04
Boeke, 2013 (43) (n=508)**	SSF(mm) at 3 years SSF(mm) and DXA derived FM(kg) at 7 years	Maternal age, weight gain, income, education, smoking, sex, ethnicity, breastfeeding	3years B-Coef {95% CI} : -1.4 {-2.7, -0.1} 7years B-Coef {95% CI} : 1.1 {-1.5, 2.1} for SSF 0.3 {-0.7, 1.3} for DXA	p <0.05 p >0.05
Mantzoros, 2009 (42) (n=588)**	SS+TR(mm) and SS/TR(mm) at 3 years of age (regression per 10ng/ml of leptin)	Maternal education, prepregnancy BMI, weight gain, gestational age, paternal BMI, sex, ethnicity, breastfeeding	B-Coef {95% CI} : -0.24 {-0.88, 0.41} for SS+TR -0.22 {-2.61, 2.17} for SS/TR	p = 0.48 p = 0.86

Table 2.4: Cord blood leptin and adiposity up to 5 years of age. Table presents data on the correlation between leptin levels and adiposity taking into consideration different covariates. FM: fat mass, FFM: fat free mass, FMI: fat mass index, SSF: sum of skinfolds, SS: subscapular, TR: triceps, VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue. */** indicates that these two studies are possibly from the same cohort followed up for different duration

2.2.5.6 Cord blood adiponectin and neonatal adiposity at birth

Pooled effect of the eight studies (78, 88, 89, 94-96, 102, 105) (735 pregnancies) revealed a weak positive correlation between cord blood adiponectin and neonatal FM ($r = 0.201$; 95% CI: 0.125, 0.277; Figure 2.7). Heterogeneity between studies was low ($I^2=26.4\%$, $p=0.17$) and a random effects model was used. Pooled mean adiponectin at birth was $25.6 \mu\text{g/mL}$ [95% CI: 16.5, 24.76; $I^2=99.77\%$, $p<0.001$] (77, 88, 89, 105).

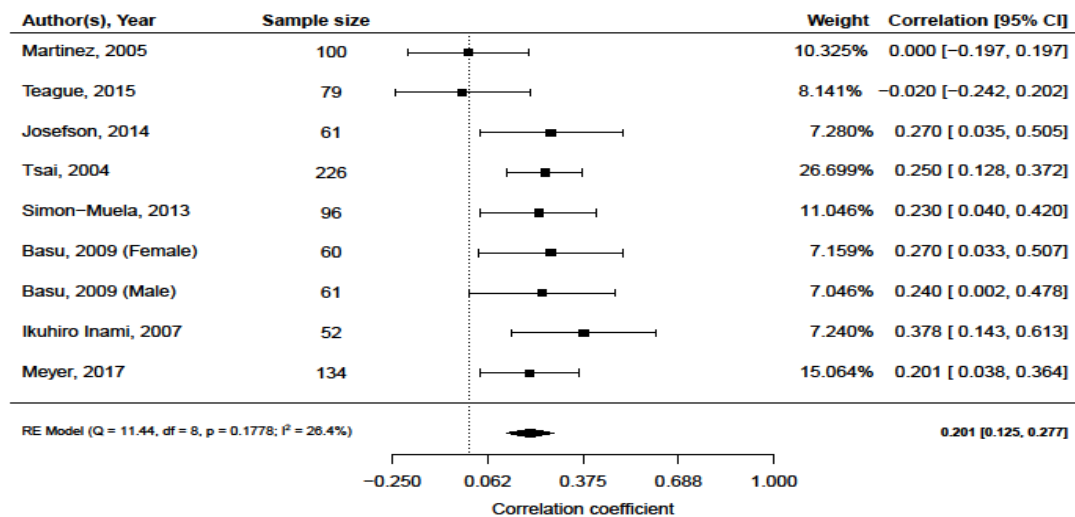


Figure 2.7: Forest plot showing the pooled correlation between cord blood adiponectin and fat mass at birth

2.2.5.7 Cord blood adiponectin and adiposity up to 5 years of age

Studies included presented inconsistent results regarding the relationship of cord blood adiponectin with weight gain and adiposity at different age groups. Teague et al (88) ($n=89$), studying HMW adiponectin, showed a weak positive correlation with both weight gain ($r=0.22$, $p=0.004$) and total adiposity ($r=0.3$, $p=0.004$) at 1 month of age. In contrast, cord adiponectin negatively predicted adiposity ($r=-0.38$, $p<0.05$) in 36 African American infants at 3 months of age (214).

Mantzoros et al (42) ($n=588$) found that cord blood adiponectin negatively predicted weight at 6 months ($r=-0.1$, $p=0.04$) but not at 3 years of age ($b=0.002$, $p=0.98$). Applying multiple regression models for maternal, paternal and offspring characteristics showed that cord adiponectin is not related to total adiposity ($b=0.42$, $p=0.12$) but positively predicts central adiposity at 3 years of age ($b=2.01$, $p=0.04$). Meyer et al (105) examining both total and HMW adiponectin found a weak positive correlation with total adiposity at 3 years of age ($b=0.04$, $p=0.04$) which did not persist to the fifth year of life ($b=0.02$, $p=0.5$;

Table 2.5). As these studies used different covariates to adjust for the results, a meta-analysis was not performed.

2.2.5.8 Publication bias

Publication bias was assessed via funnel plot only for the leptin group as the minimum requirement for ten studies per meta-analysis was not met in the adiponectin group. Application of Egger's test did not reveal any asymmetry of the funnel plot, indicating no evidence of publication bias (Figure 2.8).

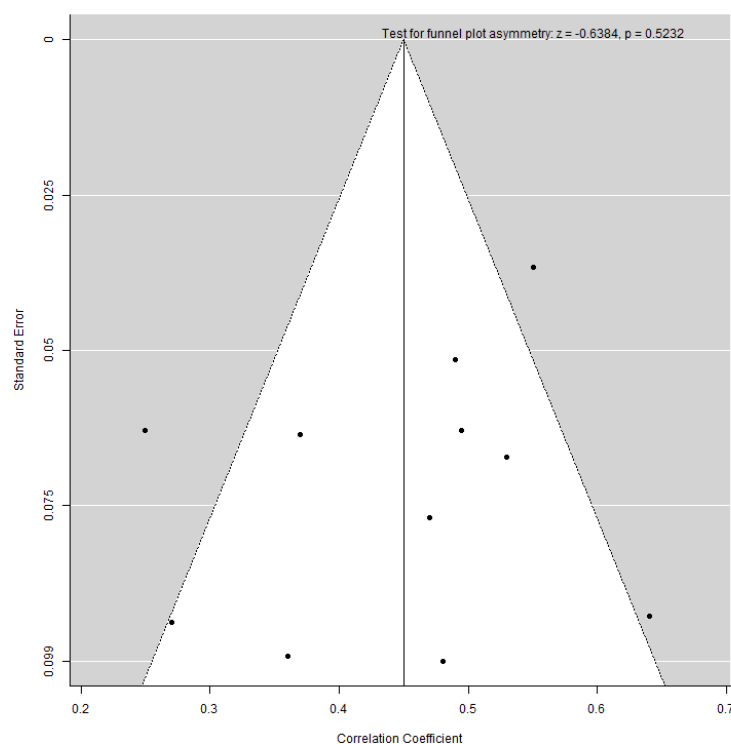


Figure 2.8: Funnel plot examining heterogeneity in the cord blood leptin and fat mass at birth group. Results suggest no evidence of publication bias

Study (Sample size)	Cord adiponectin ($\mu\text{g/ml}$) to	Adjusted for	Results	P value
Meyer, 2017 (105) (n=90)	%FM at 3 and 5 years VAT(cm^3) and SAT(cm^3) at 5 years	Maternal BMI, gestational weight gain, pregnancy duration, sex, breastfeeding	B-Coef {95% CI] %FM at 3: 0.21 {0.06, 0.35] %FM at 5: 0.08 {-0.10, 0.27] VAT: 1.57 {-2.20, 5.34] SAT: 7.22 {-10.17, 24.62]	p <0.05 p = 0.36 p = 0.39 p = 0.40
Schneider, 2017 (103) (n=36)	FM(g) at 2 weeks and conditional change from 2 weeks to 3 months	2 weeks: gestational age, age at measurement, FFM 3 months: above+ 2 weeks measurement and time between measurements	2 weeks: r = 0.45 3months: r = -0.38	p <0.001 p <0.05
Teague, 2015 (88) (n=52)	%FM at 1month	Diabetic status, sex, age in days	r = 0.32	p = 0.02
Mantzoros, 2009 (42) (n=588)	SS+TR(mm) and SS/TR(mm) at 3 years of age (regression per $10\mu\text{g/ml}$ of adiponectin)	Maternal education, prepregnancy BMI, weight gain, gestational age, paternal BMI, sex, ethnicity, breastfeeding	B-Coef {95% CI] : 0.42 {-0.11, 0.95] for SS+TR 2.01 {0.09, 3.93] for SS/TR	p = 0.12 p = 0.04

Table 2.5: Cord blood adiponectin and adiposity up to 5 years of age, after adjusting for different covariates. FM: fat mass, FFM: fat free mass, SS: subscapular, TR: triceps, VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue

2.2.6 Discussion

We observed three key findings in this systematic review. First, cord leptin and adiponectin are positively associated with adiposity at birth, suggesting these are useful objective markers for adiposity. The association was stronger for leptin than adiponectin. Second, the association between leptin with adiposity seems to reverse after the first few months of life and is negatively associated with adiposity at 3 years of age. Third, cord adiponectin levels seem to be positively associated with adiposity in young children, but the data is limited.

2.2.6.1 Leptin and adiposity

Maternal leptin cannot cross the placenta due to its high molecular weight (15). Cord leptin is primarily derived from fetal adipose tissue although it could also come from the placenta. While the majority of placental leptin drains in to the maternal circulation, small amounts enter the fetal circulation (16). Leptin levels are higher in the umbilical artery compared to umbilical vein (7). Fetal leptin is detectable as early as 18 weeks of gestation, with levels rising as pregnancy progresses, in concordance with fetal fat accumulation (17). In addition, the presence of leptin mRNA and leptin receptors in various fetal tissues implies its role as a growth factor during intrauterine development. All these findings support that cord leptin is a good marker of adiposity at birth.

Our study showed that cord leptin levels are negatively related to adiposity at 3 years of age (9, 42, 43, 104), but this association is not sustained (43, 97) at 4 and 7 years. Chaoimh et al (91) showed that cord leptin is negatively associated with adiposity gain from birth to 2 months of age, by an objective adiposity measure, ADP. In our study, pooled mean leptin level at birth was at 9.1 ng/ml (n=2242). Although, there is no reference range for cord blood leptin levels, Karakosta et al (107) showed a mean leptin value of 7.7ng/ml in a cohort of 398 healthy, full term neonates, born in Greece. Taken together, it appears that children born with higher leptin levels, may develop a 'compensatory behavior' driven by the anorexigenic effect of leptin in early postnatal life which lasts up to 3 years of age. In order to maintain a positive energy balance and enhance vital organ development, leptin's full metabolic effect is not exerted before the second post natal week (108). The above observation could potentially explain the positive correlation between cord leptin and adiposity during the first weeks of life, as described by Schneider et al (103) and Teague et al (88).

The above conclusion is also supported by evidence from animal studies. Leptin plays an important role in brain development. Human hypothalamus develops predominantly during the pre-natal period. ARC, the major site for energy regulation, develops after 34 weeks of gestation but further changes take place in the early post-natal life. Independent of FM accretion, leptin surge happens in the immediate post-natal period, which is critical for the development of projections from the ARC to paraventricular hypothalamic nuclei (109). Ob/ob mice have impaired projections, an effect which can be reversed by early life leptin administration, whereas leptin administration in adulthood has no effect, implying a tight window in leptin's neurotrophic action (28). Timing, amplitude and duration of the leptin surge are critical for the development of adverse metabolic behaviors. Obese dam's offsprings (prolonged leptin surge) (110) and growth restricted pups (low amplitude surge) (111) are characterized by impaired ARC projections and neurogenesis of neurons with orexigenic phenotype, resulting in hyperphagia and obesity which can be traced to adulthood. Kirk et al (221) studying overfed vs. control dams in pregnancy and lactation tried to investigate the 'transmission of the obesogenic trait'. Overfed pups had prolonged and amplified leptin surge and were heavier at birth. Weight and leptin levels were similar 30 days post birth (compensatory behavior) but five weeks later overfed pups developed hyperphagia resulting in increased weight and FM in adulthood (adverse effect of impaired leptin surge). Leptin administration at day 30 and 90 had no effect on overfed pups and was accompanied by lower neuronal activation and impaired ARC projections (leptin resistance). The study clearly demonstrated that leptin resistance starts prior to hyperleptinaemia and obesity. Thus, in our study, raised leptin levels at birth may have adversely programmed the hypothalamus (via impaired leptin surge), with effects becoming evident after third year of life. The initial negative correlation between cord leptin and adiposity may be due to the anorexigenic effect of leptin, followed by leptin resistance resulting in hyperphagia and increased adiposity. In the present systematic review, Meyer et al (215) and Boeke et al (155) used direct measures of adiposity (MRI, DXA) to assess the association between cord leptin and adiposity beyond the third year of life. Both studies demonstrated a trend for cord leptin to negatively predict adiposity at 3 years of life, which was converted to a positive association (although not reaching statistical significance) by the age of 5 and 7. Boeke et al (155) also showed that serum leptin at 3 years of age positively predicts adiposity at 7, results consistent with leptin resistance.

2.2.6.2 Adiponectin and adiposity

Adiponectin levels increase by 20-fold from midgestation to term (112). Cord levels are not related to maternal adiponectin (46, 47) and placental production is not yet confirmed (60, 61). Sivan et al (62) showed that cord blood adiponectin levels were similar to those four days post partum, confirming the independent fetal production. The inverse correlation between adipose tissue and adiponectin observed in adult population is not present during the early stages of life. Evidence suggests that the shift from “positive to negative” correlation between adiponectin and FM occurs around school entry age (71). Multiple sites of adiponectin expression other than adipose tissue during fetal life (67), higher brown adipose tissue (73) and subcutaneous to visceral fat ratio of neonates (74) and the inhibition of adiponectin expression from inflammatory cytokines produced by hypertrophic adult adipocytes (75) could potentially explain the different metabolic profile between neonatal and adult life.

The present report is unable to demonstrate any correlation (negative or positive) between cord adiponectin and future adiposity. Small sample size, different methods to measure adiponectin (ELISA, RIA) and different multimeric forms examined may contribute to the inconsistent results. Simpson et al (113) recently reviewed the association between adiponectin and adiposity at 9 and 17 years of age. Results showed no correlation with adiposity at 9 years and a positive correlation at 17 years with a very small effect size (b coef: 0.02, $p < 0.05$). Major limitations of the study were the big loss to follow up and the small percentage of children with obesity/overweight (sample not representative of most populations) which could have potentially attenuated any associations.

2.2.6.3 Strengths and weaknesses

To our knowledge, our systematic review is the first to assess the correlation of cord blood adipocytokines with adiposity at different time points. However, while it had reasonable sample size to assess the independent associations of leptin and adiponectin with adiposity at birth, it did not have adequate sample size for early childhood. Publication bias was examined and selection bias was minimised by using quality assessment tools. As this review included observational cohorts and cross sectional studies, the main limitation is that correlation results do not necessitate causality. We also acknowledge that in the majority of the studies FM was derived from the sum of skinfolds and direct measures of adiposity would have minimized intra and inter subject variability. Additional studies on the long term effects of adipocytokines are required to

understand the metabolic pathways of intrauterine programming and how early life biomarkers could predict future adverse metabolic profile.

2.2.6.4 Conclusion

The present meta-analysis reveals an association between cord blood adipocytokines and body fat at birth. Cord blood leptin inversely predicts adiposity up to 3 years of age whereas adiposity at 5 years seems to be positively predicted by cord levels. Results for adiponectin remain controversial and further longitudinal studies are required in order to draw safe conclusions

2.3 Closing Remarks

Up to date knowledge on the mechanisms underpinning intrauterine programming remains limited (114), and the 'gold programming factor' is yet to be identified (115). In order to understand the origins of obesity and metabolic diseases we need to investigate growth in terms of body composition. With the National Health System under time and financial pressure, weight and BMI seem to be the best methods of assessing obesity. On the other hand, both these anthropometric measurements are not reliable during the early stages of life, as they cannot differentiate between fat and fat free mass. Moreover, it is more likely that during these developmental stages increased BMI reflects lean rather than adipose tissue accumulation. It is also well described that universal BMI "cut offs" cannot be applied as different ethnic groups have more fat for a given BMI. Skinfold thickness is an alternative which provides more information on the actual body composition. Results can be obtained in a non-interventional, non time-consuming way but are prone to large variations, within and between subjects. Direct methods (DXA, MRI, ADP) of assessing body composition allow for precise, objective measurements and can be used to create growth charts and identify individuals at high metabolic risk in order to prevent development of adverse outcomes. Measuring body composition in neonates is technically challenging and available methods are not yet widely accessible, mainly due to cost and time required for each test. Each technique is based on theoretical assumptions, thus data obtained from different methods cannot be combined to create universal standards (84, 116). Utilizing cord blood biomarkers to predict future adiposity is a promising field in understanding fetal programming and intervening in the early stages of life. Cord blood samples can be obtained easily in a non- invasive way with minimal training for the healthcare professionals. The high cost of the available adipocytokines' assays remains one of the biggest challenges to overcome. Stronger

evidence of their predictive value and broad universal screening would reduce the cost of testing. In addition, proving the utility value of these assays will also allow technology companies to create point of care kits at low cost, which will enable such tests can be performed easily even in resource-poor settings. Identifying high risk neonates from a simple blood test may be the future approach in tacking and preventing childhood obesity.

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Chapter 3

The impact of Gestational Diabetes Mellitus on infant body composition

3.1 Introduction

Exposures during antenatal and early post natal life can metabolically imprint the offspring with long lasting effects. Birthweight is a rough estimate of body composition which doesn't precisely reflect nutritional status during intrauterine life. In order to understand the mechanisms underpinning the "intrauterine origins of metabolic diseases" detailed data on neonatal body composition is required.

Gestational diabetes mellitus rises in parallel with adult obesity, constituting one of the commonest metabolic problems in pregnancy. Pregnancies complicated by diabetes face short and long term adverse consequences, for both mother and the offspring. Despite convincing evidence suggesting the beneficial effects of maintaining normoglycaemia on short term outcomes, data reveals that glucose control on its own is not adequate to prevent future offspring obesity. The exact reason remains unknown and various mechanisms (epigenetics, environmental exposures, maternal dyslipidaemia) have been proposed. Taking a closer look at the body composition evolution during early life may provide insight on the pathogenesis of obesity and metabolic diseases. In this regard, we performed a longitudinal, observational study looking at the effects of maternal GDM on offspring body composition up to 5 months of age.

3.2 The role of maternal adverse/risk outcomes on offspring's body composition: Prospective, longitudinal cohort study

3.2.1 Hypothesis

GDM is linked to increased risk of offspring obesity, impaired glucose tolerance and metabolic syndrome. This association persists despite optimal glycaemic control. We hypothesize that events occurring during prenatal, antenatal and immediate postnatal life metabolically imprint offspring's health. This adverse effect may be potentially reflected in altered infant body composition.

3.2.2 Research Questions

- Is maternal GDM linked to altered body composition at birth and up to 5 months of age?

3.2.3 Outcomes and criteria

- Assess differences in body composition (fat and fat free mass) in newborns of mothers with (case) and without (control) GDM

3.2.3.1 Inclusion criteria

- All pregnant women between 18-45 years who delivered at George Eliot Hospital
- All pregnant women between 18-45 years who had their antenatal care at George Eliot Hospital and had a home birth

3.2.3.2 Exclusion criteria

- Congenital abnormalities, identified either during anomaly scan or immediately post delivery

3.2.4 Study design

3.2.4.1 Sample size calculation

We estimated the sample size for the outcomes based on 5% significance. We calculated that a minimum of 40 neonates in the GDM group would provide 80% power to detect a mean difference of 10% in total adipose tissue between cases and controls. We considered these differences to be clinically significant as they are similar to those between preterm and healthy term infants (47); the former group is known to have higher risk for future metabolic diseases.

At GEH (recruitment site) 15% of pregnancies are complicated by GDM. Allowing for a 30% drop out from the follow up studies, we aimed to recruit at least 400 participants in order to achieve the required targets.

3.2.4.2 Application and Approvals

In order to start the research project author, who is also the co-investigator of the study, had to sign an honorary contract with GEH allowing him to approach NHS patient-service users for research purposes (Appendix 6). Project was then discussed with local Research and Development (R&D) office and details on duration, expected level of participation, equipment and infrastructure required were provided. After thoughtful

consideration GEH R&D department consented for the research project to be accommodated at GEH premises (Appendix 7).

Following the completion of a Health Research Authority (HRA) Statement of Activities template a co-sponsorship agreement was signed between the University of Warwick (UoW; Lead Research Sponsor) and GEH (Co-sponsor) setting the terms for their respective duties. In specific, UoW held the Public and Products Liability insurance, Clinical Trial insurance and had the PEAPOD ownership. It was also responsible to ensure all the research staff who had access to the GEH facilities had all the up to date research training. GEH was responsible for all the study related clinical procedures, maintaining standard operating procedures, updating protocols, training of staff, maintaining study related documents in safe areas as per Good Clinical Practice (GCP) guidelines and making all the above available for audit and inspection as necessary .

As the research project took place and involved users of the NHS England, HRA approval was mandatory. The process involved the following steps (Figure 3.1):

- I. Completion of the portfolio application form, found in the Integrated Research Application System (IRAS), for inclusion of the study on the National Institute for Health Research – Clinical Research Network (NIHR-CRN) portfolio
- II. Completion of the Research Application form found in IRAS, stating the nature and the process of the research project
- III. Uploading of all research relevant documents to the IRAS database, including protocol, patient information sheet, consent form, questionnaires, study flow chart, sponsorship and equipment agreements, honorary contract and investigators' curriculum vitae
- IV. Booking of an “application review” date with Research Ethics Committee (REC), examining the ethical aspects of the study
- V. Application for HRA approval, assessing the governance and legal compliance of the study

Study received a REC favorable opinion and was fully approved by HRA (Appendix 8 and 9)

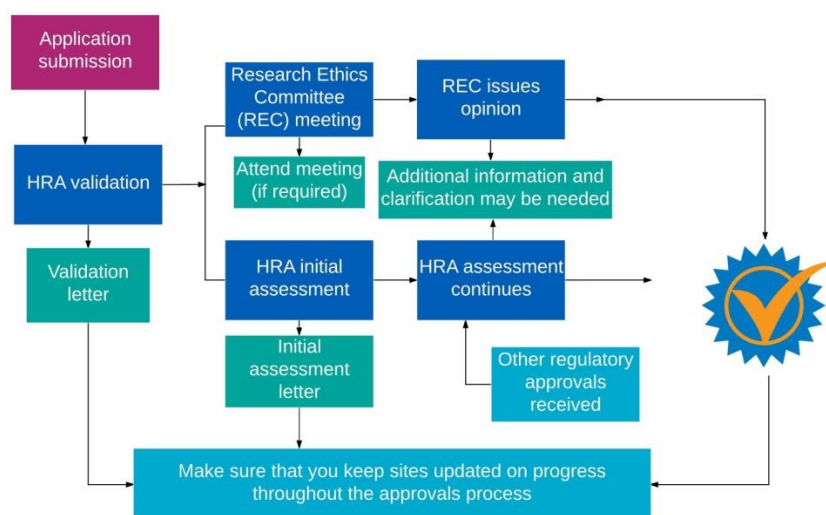


Figure 3.1: Steps in gaining HRA approval

3.2.4.3 Preparing for the study initiation

One of the most challenging parts in setting up the study was finding the room to host the PEAPOD machine. The room had to be close to maternity ward (for newborn's measurement), accessible to parents (measurements during follow up visits), spacious (due to PEAPOD size) and with relatively stable ambient temperature (PEAPOD requirements). After considering available options it was decided that the PEAPOD room would be based on maternity ward, next to the paediatric assessment room, allowing easy access to mothers and newborns in the immediate postpartum period. For the follow up appointments it was decided that participants should report to maternity main reception and accompanied to the PEAPOD room by a member of the research team. It was instantly noted that the geographical direction of the room did not allow stable and within target range temperatures during summer period (hot weather), therefore after liaising with hospital Estates services a portable Air Conditioning unit was installed in the room.

The current research project was part of a larger longitudinal study (PEAPOD study), with a predicted recruitment of 3,000 participants over a 5 years period. In order to maintain high quality standards and keep up with the requirements of the study a fully qualified research team was formed which included two research midwives (Karen Shorthose, Judith Plester), one research associate (Gail Pounder), one research bioscientist (Ilona Goljan), a statistician (Yonas Weldeselassie) and a research coordinator (Amitha Gopinath). Professor Ponnusamy Saravanan was the Chief

Investigator and the author was the Co-Investigator. Author was responsible for the study set up, organization and delegation of job roles to the team members. The responsibilities of each member were stated in the study delegation logbook (Appendix 10).

Instructions on operating, troubleshooting and maintaining PEAPOD were provided by a company's (COSMED) representative to the research team. Members responsible for performing maternal and neonatal measurements (author and midwives) had further training on obtaining anthropometric measurements. Anthropometry protocol of the WHO Multicentre Growth Reference Study (MGRS) and INTERGROWTH-21st anthropometry handbook (48) were used as reference techniques. Audiovisual material was also used for better understanding of the process in an attempt to minimize intra- and inter-observer variability.

In order to raise awareness of the study among the public and midwifery, posters were used in General Practice (GP) waiting rooms, community rooms and libraries. The study was also advertised on social media through GEH account. Advertising material contained brief information about the study and advised potential participants to contact the research team for more information (Appendix 11). Three multidisciplinary team forums were organized in the local areas and the study protocol was presented in detail. Author working in collaboration with Primary Care, Diabetes & Endocrinology Local Research Networks and the research team were responsible for organizing these meetings.

For “out of hours” deliveries, the participation of non-research midwives in collecting cord blood samples was necessary. After approaching the Head of Midwifery and explaining the purpose and the structure of the study we got approval for training midwives in the delivery unit on how to collect, centrifuge and store cord blood samples. Posters providing a brief description of the study and detailed instructions on how to perform cord blood collection and storage were placed in the delivery unit and sluice room (Figure 3.2). Author and the research bioscientist performed regular quality inspections and provided training to any new member of the staff.



DELIVERY ALERT FOR MIDWIVES

Please ring the PEAPOD study team for ALL participants admitted for delivery.


Tel No: 02476865358 or 02476153592

(leave a message on answer phone if out-of-hours)

Dear Delivery team,

This lady is a **PEAPOD study participant**. She has consented for collection of delivery samples which are vital for our research. We would be extremely grateful for your help with the same.

- **Working Hours:** We will collect necessary bloods, placenta and cord samples.
- **Out of hours :** Please could you collect cord blood as a priority as below.
 1. Fill 2 EDTA (purple) and 2 PLAIN (red/yellow) tubes provided in this delivery pack given to the mother (minimum 1 tube of each colour)
 2. Place bottles in labelled blood form provided. Record date and time of collection.
 3. Ring Lab technician and send to lab within 30 mins for urgent processing.
 4. If participant does not have a delivery pack in possession, blood forms and stickers are also available in the maternity section of the medical notes



Thank you for helping with the **PEAPOD Study!**

Dr P Saravanan and team.

George Eliot Hospital 

Figure 3.2: Delivery alert for midwives. Contact details for research team and instructions for collecting the samples are clearly indicated

3.2.4.4 STUDY INITIATION AND VISITS

The prospective observational case-control study involved pregnant women planning to deliver at GEH and involved four study visits (Figure 3.3). Study was officially launched on the 13/02/2017. Inclusion and exclusion criteria are described in chapter 1.

Role of maternal adverse (risk) outcomes and ethnicity on offspring body fat content in White Europeans and South Asians

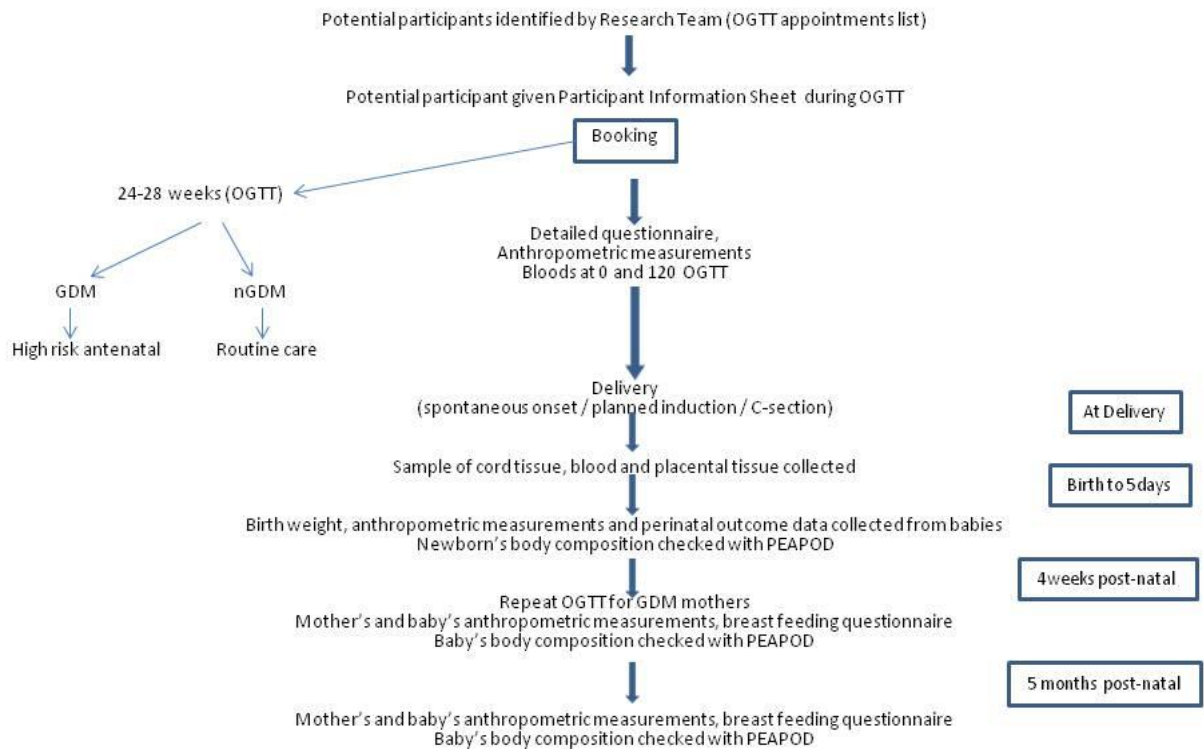


Figure 3.3: Flowchart of the study detailing recruitment and follow up visits

3.2.4.4.1 Visit 1/ Recruitment

Vast majority of the participants were recruited during their OGTT (24-28 weeks gestation). OGTT appointment lists were screened by the research team one week in advance identifying potential participants for the study. On arrival to the Antenatal ward mothers were approached by a member of the research team and were provided with information about the study. A participant information sheet was given to all potential candidates (Appendix 12). Main priority during the consent was to ensure that mothers had capacity to make a well informed decision. It was also always highlighted that participation was entirely voluntary and decision not to participate would not compromise their medical/obstetric care. Mothers who were happy to participate were recruited at the same appointment (Appendix 13). For those requiring more time to consider their participation an extra visit was offered (none of the participants involved in the current thesis/study required the extra visit). Mothers who expressed their interest in participating (recruited by publicity; posters, adverts) but did not belong to the high metabolic risk

group, thus not requiring an OGTT, were recruited during their booking visit (12 weeks) in the Antenatal clinic (during this thesis/study only two participants were recruited in early pregnancy).

During the recruitment visit an additional 10ml were withdrawn with each blood sampling of the OGTT (OGTT₀ and OGTT₁₂₀). Blood samples were pseudoanonymised with a unique study ID number and immediately centrifuged. Using pipettes, samples were further divided to four aliquots containing serum, four containing plasma, one blood and one buffy coat (Figure 3.4). All aliquots were then placed in an ultra low freezer at -80°C, where they were stored until further analysis.

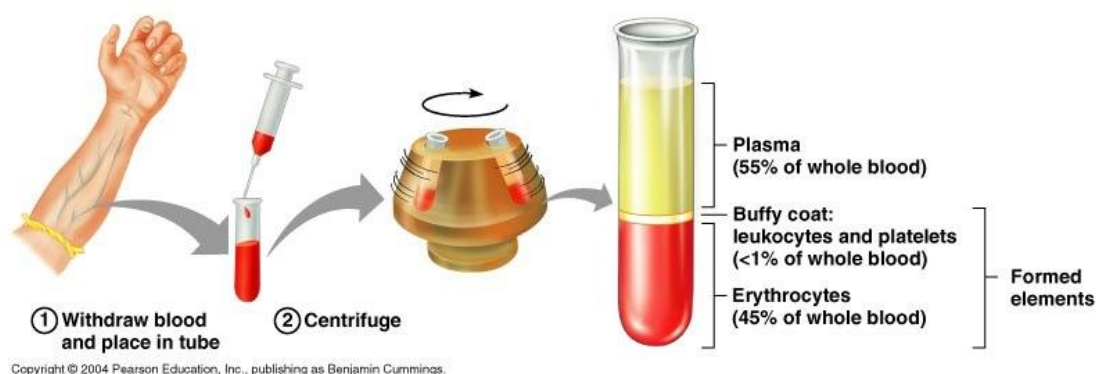


Figure 3.4: Centrifugation and blood fractionation (Copyright 2004, Pearson Education, Inc., Benjamin Cummings)

During the two hours waiting between the OGTT sampling, basic maternal anthropometric measurements were obtained, including height, weight, abdominal circumference and skinfold thickness (Appendix 14). Participants were also asked to complete questionnaires assessing their physical activity, well-being, socioeconomic status, anxiety and depression levels (Appendix 15). Ethnicity was self-selected by participants and in cases of migrant population further information on the ethnicity of their parents and grandparents was collected. The following categories were available for selection: White British, South Asian, African and “other”. A “delivery pack” which included two blood bottles for cord blood sample collection and an information note to the midwives (identifying mother as a study participant) was handed over to all participants. A sticker with the study logo was placed on the right top corner of the maternal notes, enabling prompt identification of study participants. A copy of the consent form was placed in the participant’s medical/ obstetric notes.

Following the OGTT results, participants were further categorized to GDM and non-GDM group (controls) based on the NICE 2015 criteria (49). GDM group had regular reviews (every one or two weeks) in the antenatal clinic, as dictated by NICE and hospital guidelines. Dietary and exercise advice were provided and target blood glucose levels were set at $<5.5\text{mmol/l}$ premeal and $<7.8\text{mmol/l}$ 1hr postprandial. Diet, metformin and insulin were used to achieve glycaemic targets. Fetal growth was assessed by ultrasound scans every 4 weeks or earlier if clinically indicated. Controls had standard antenatal care.

3.2.4.4.2 Visit 2/ Delivery

Research team kept a logbook of estimated delivery date of each participant. Furthermore Delivery Unit capacity was checked on a daily basis for names participating in the study. Midwives on the Delivery Unit, were notifying research team about the delivery of a participant on a daily basis. In case of “within working hours” deliveries a member of the research team performed cord blood centrifugation whereas staff midwives centrifuged and stored cord samples for “out of hours” deliveries. Samples were then allocated to four aliquots containing serum, four containing plasma, one blood and one buffy coat and were stored at -80°C . Women who had a home birth were still eligible to continue in the study, even without having cord blood samples collected.

Author or one of the research midwives approached participants within five days after delivery, optimally during their stay in the Maternity ward, checking if they were still happy to continue in the study. Birth weight and anthropometric measurements (chest and waist circumference, length, triceps and sub scapular skinfold thickness) were collected for offsprings whereas mothers had their weight and skinfold thickness measured. Perinatal outcome data (mode of delivery, complications/birth injury, genetic anomaly, Apgar score, head circumference) was collected from obstetric notes. For pregnancies complicated by GDM, mode of treatment and last HbA1c were also acquired. Offsprings had their body composition assessed using PEAPOD (Appendix 16). Principles and methods of assessing skinfold thickness and body composition using PEAPOD have been previously described.

After completion of the assessment participants were asked again if they were happy to continue with the follow up visits. Appointments were booked for a day and time convenient to them (weekdays, working hours) and a text reminder was sent one week before the next visit.

3.2.4.4.3 Visit 3 (4 weeks post partum) and Visit 4 (5 months post partum)

Participants were asked to attend their follow up visits at 4 weeks and 5 months post partum. Offsprings had anthropometric measurements (length, triceps and sub scapular skinfold thickness) and body composition assessment via PEAPOD (Appendix 17). Mothers had their weight checked and were asked to complete two questionnaires, regarding feeding mode and sleep quality (Appendix 18). Mean duration of each visit was ten minutes.

The breastfeeding questionnaire was used to categorize feeding patterns to predominantly breastfeeding, predominantly formula feeding and mixed feeding by addressing mode and duration of feeding. The “Pittsburgh Sleep Quality Index” (PSQI) questionnaire was used to differentiate “poor from good” sleep quality by measuring subjective sleep quality, sleep latency, sleep disturbances, daytime somnolence and use of sleeping medication during the last one month.

3.2.4.5 Data extraction

After study completion all the data was converted into electronic form. For analysis, no personal identifiable data was required. Hence this was carried out using the anonymous data with the link secured stored in NHS and University computers. Anthropometric measurements (weight, length, body's fat content) were transferred from the PEAPOD database to password protected computers. Demographic, maternal and neonatal characteristics documented in paper report forms were transferred to an electronic database and linked to the pre-mentioned PEAPOD information. All analysis took place either at GEH or at UoW using laptops/computers which remained locked in password protected areas. Only research staff had access to these areas.

In order to explore the pathophysiology of GDM in depth and its effects on fetal development, GEH electronic imaging database was used to extract information from ultrasound anomaly scans at 20 weeks. Data on estimated fetal weight (EFW), head (HC)-abdominal (AC) circumference and femur length (FL) was retrieved. Hadlock formula was used to calculate EFW (50).

Due to the nature of study, the participants' personal data will be kept for 15-20 years after the completion of the study, as there may be follow up studies on the mothers and

the offspring. This data will be kept in a separate file and stored under strict secure conditions as mentioned above.

3.2.4.6 Statistical analysis

Data was analyzed using SPSS version 22. Statistical analysis was performed using “t test” or “Mann-Whitney U test” depending on data distribution (parametric or not). Independent one-way and mixed multi-factorial ANOVA was used to compare data across three or more independent variables. Chi-squared test was used to compare categorical data. For the primary outcome linear regression analyses was used to assess whether GDM independently predicts the risk of increased adiposity in the offspring. Results were adjusted for maternal and neonatal characteristics. As %FM adjusts only for body weight, statistically optimal indices were used to adjust fat and fat free mass for infant size. These were FM/length (cubed) and FFM/length (cubed) in the neonatal period (visit 2); FM/length (squared) and FFM/length (squared) for the follow up visits (visit 3 and 4) (51). Risk factors for GDM were considered as covariates and their independent effect on body composition was assessed. The author performed all statistical analyses. The accuracy of the methods and results were reviewed by the Chief Investigator’s institution and senior statistician.

3.2.5 Results

508 mothers were approached from February 2017 to July 2018 at George Eliot Hospital NHS Trusts, Nuneaton, UK. 18% refused to take part in the study, whereas 417 were happy to be recruited and signed the consent form. The main reasons for refusing to take part in the study were lack of time (45%), lack of interest in participating into research (20%) and hesitation about the use of PEAPOD (35%). Participants completed their follow up visits by February 2019, allowing data to be analyzed and presented before August 2019 (thesis submission). 255 participants and their offspring undertook assessment at birth, 220 attended visit 3 and a total of 192 participants completed all four visits. A detailed flowchart of the study is presented in Figure 3.5. In order to assess the effect of intrauterine environment on body composition, data from all newborns measured at birth (n=255) was considered. The evolution of body composition during infancy was assessed using data from infants who completed all four visits (n=192). Maternal, fetal and neonatal characteristics were similar between the group who completed only visit 2 and those who completed all study visits.

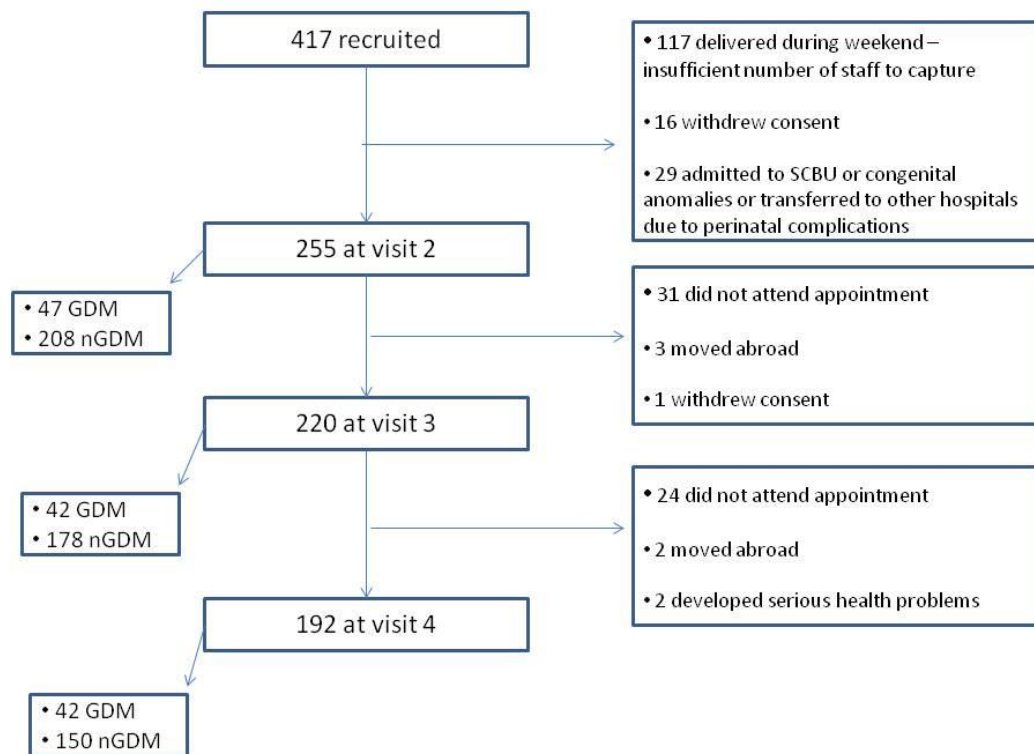


Figure 3.5: Flow-chart of the study showing participants' recruitment and attendance to follow up visits. A total of 192 participants were included for final analysis; visit 2: birth, visit 3: 4 weeks post partum, visit 4: 5 months post partum

3.2.5.1 Maternal Characteristics

Following the 75gr OGTT at 28 weeks, participants were categorized into GDM (n=47) and nGDM (n=208) groups. GDM group had higher BMI (32.4 ± 5.5 vs. 29.5 ± 6.9 kg/m², $p < 0.01$) at booking visit (12 weeks gestation), lower gestational weight gain (3.851 ± 2.687 vs. 7.208 ± 4.375 kg, $p = 0.02$) and were delivered 9 days earlier ($p = 0.02$). Maternal age and ethnic variation were similar between the two groups (Table 3.1).

The GDM group achieved good glycaemic control, with a mean (SD) third trimester HbA1c of 34.9mmol/mol (4.3). 23 participants were diet controlled, 13 were treated with metformin and 11 required the addition of insulin to achieve glycaemic targets.

	GDM (n=47)	nGDM (n=208)	p value
Maternal Age (years)	32.3 (5.8)	32.1 (5.4)	0.8
Ethnicity	40 White Europeans 6 Asians 1 African	178 White Europeans 29 Asians 4 Africans	0.9
BMI (kgr/m²)	32.4 (5.5)	29.5 (6.9)	<0.01
GWG (kgr)	3.851 (2.687)	7.208 (4.375)	0.02
Gestational Age (weeks)	38.0 (1.1)	39.3 (2.4)	0.02
Delivery	18 (38.1%) CS 29 (62%) normal	56 (27%) CS 152 (73%) normal	0.1

Table 3.1: Maternal characteristics. The GDM group had higher BMI, lower gestational weight gain and was delivered earlier. $p < 0.05$ is considered statistically significant

3.2.5.2 Fetal characteristics based on 20 weeks scan

Gestational age was similar between the two groups during fetal scan (20.3 ± 0.6 vs. 20.3 ± 0.4 weeks, $p=0.6$). GDM group fetuses were found to have higher EFW (372.1 ± 52.3 vs. 355.8 ± 38.5 grams, $p=0.01$) and larger HC (17.8 ± 0.8 vs. 17.6 ± 0.7 cm, $p=0.01$). There was no difference in AC or FL between groups (Table 3.2). After adjustment for maternal BMI and age, gestational age and gender of the fetus, EFW and HC remained significantly higher in the GDM group.

	Fetal scan		
	GDM (n=47)	nGDM (n=208)	p value
Gestational age (weeks)	20.3 (0.6)	20.3 (0.4)	0.6
EFW (grams)	372.1 (52.3)	355.8 (38.5)	0.01
HC (cm)	17.8 (0.8)	17.6 (0.7)	0.01
AC (cm)	15.8 (1.2)	15.6 (0.8)	0.08
FL (cm)	3.3 (0.2)	3.2 (0.2)	0.4

Table 3.2: Fetal data during 20 weeks scan. GDM fetuses tended to be heavier with higher head circumference

3.2.5.3 Neonatal Characteristics at birth (visit 2)

Mean time (SD) of neonatal body composition measurement was 42 (9.8) hours after birth for both groups. GDM group neonates had lower birth weight (3.078 ± 0.407 vs. 3.322 ± 0.514 kgr, $p < 0.01$) and were shorter (48 ± 2 vs. 49 ± 2 cm, $p = 0.02$). There was no difference in gender between groups. Babies born to mothers with GDM were born slightly earlier than the non-GDM (38.0 ± 1.1 vs. 39.3 ± 2.4 ; $p = 0.02$; table 3.3).

PEAPOD assessment revealed similar %FM ($9.9 \pm 4.4\%$ vs. $10.7 \pm 4.1\%$, $p = 0.7$) and FM (0.317 ± 0.171 vs. 0.369 ± 0.171 kgr, $p = 0.06$) between the two groups. GDM group neonates had lower FFM (2.761 ± 0.295 vs. 2.954 ± 0.405 kgr, $p < 0.01$). Adjustments for neonatal size (FMI, FFMI) didn't alter the results (Table 3.7). After adjustments for maternal BMI, maternal weight gain and gestational age at delivery, FM results between the two groups remained unchanged whereas the FFM difference attenuated and became non significant (Table 3.4).

Visit 2 (birth)	GDM (n=47)	nGDM (n=208)	P VALUE
Gender	23 Males 24 Females	93 Males 115 Females	0.6
Birthweight (kgr)	3.078 (0.407)	3.322 (0.514)	<0.01
Gestational age	38.0 (1.1)	39.3 (2.4)	0.02
Length (m)	0.48 (0.02)	0.49 (0.02)	0.02
%FM1	9.9 (4.4)	10.7 (4.1)	0.2
FM (kgr)	0.317 (0.171)	0.369 (0.171)	0.06
FFM (kgr)	2.761 (0.295)	2.954 (0.405)	<0.01
FMI (kgr/m³)	2.6 (1.2)	2.9 (1.2)	0.1
FFMI (kgr/m³)	22 (4.7)	23.9 (2.9)	<0.01

Table 3.3: Neonatal characteristics at birth. GDM neonates were shorter but also lighter. The difference in birthweight was driven by lower FFM whereas FM was similar; $p < 0.05$ is considered statistically significant

a)

Visit 2 (Birth)	Model 1			Model 2	
	B coef.	Sig.		B coef.	Sig.
%FM	-0.790	0.2		-0.639	0.3
FM (kgr)	-0.052	0.06		-0.039	0.1
FFM (kgr)	-0.193	<0.01		-0.119	0.06

b)

Model	B	SEE	Beta	p value
GDM	-0.119	0.061	-0.118	0.06
BMI	0.008	0.004	0.130	0.07
GWG	0.008	0.003	0.185	0.02
GA	0.063	0.010	0.378	<0.01

Table 3.4: **a)** Regression of neonatal body composition between the two groups; Model 1: unadjusted, Model 2: adjusted for maternal BMI, maternal weight gain and gestational age **b)** Regression analysis of fat free mass (FFM) at birth adjusting for maternal BMI, maternal gestational weight gain (GWG) and gestational age (GA) revealing gestational weight gain and gestational age as independent predictive factors.

3.2.5.4 Body composition at visit 3 (4 weeks)

Mean age (SD) at the second assessment was similar for both groups (GDM: 32 ± 3 days; nGDM: 33 ± 6 days, $p=0.6$). During visit 3 GDM group infants remained shorter (53 ± 2 vs. 54 ± 2 cm, $p=0.1$) with lower total body weight (4.206 ± 0.468 vs. 4.388 ± 0.639 kgr, $p=0.08$). PEAPOD assessment revealed that both %FM ($16.7 \pm 5.3\%$ vs. $17.6 \pm 4.7\%$) and FFM (3.487 ± 0.302 vs. 3.602 ± 0.471 kgr) remained numerically lower in the GDM group, although not reaching statistical significance ($p>0.05$; Table 3.5). Results remained unchanged after adjustment for infant size, maternal characteristics and feeding mode.

Visit 3 (4 weeks)	GDM (n=42)	nGDM (n=150)	P VALUE
Age (Days)	32 (3)	33 (6)	0.6
Weight (kgr)	4.206 (0.468)	4.388 (0.659)	0.08
Weight gain (kgr)	1.086 (0.396)	1.061 (0.453)	0.7
Length (m)	0.53 (0.02)	0.54 (0.02)	0.1
%FM	16.7 (5.3)	17.6 (4.7)	0.2
FM (kgr)	0.718 (0.269)	0.785 (0.285)	0.1
FFM (kgr)	3.487 (0.302)	3.602 (0.471)	0.1
FMI (kgr/m ²)	2.5 (0.9)	2.6 (0.8)	0.4
FFMI (kgr/m ²)	12.1 (1.1)	12.1 (1.1)	0.9
Feeding mode	n (%)	n (%)	0.4
Breastfeeding	15 (35.7)	55 (36.6)	
Formula-feeding	23 (54.7)	70 (46.6)	
Mixed	4 (9.5)	25 (16.6)	

Table 3.5: Infant characteristics and feeding mode at visit 3. There was no between groups difference noted.

3.2.5.5 Body composition at visit 4 (5 months)

Offspring of both groups had the same age during their last assessment. GDM group demonstrated a more rapid growth with higher weight gain between visits 3 and 4 (3.226 ± 0.694 vs. 3.078 ± 0.670 kgr, $p=0.2$). As a result weight at visit 4 was similar between the 2 groups (GDM: 7.432 ± 0.825 vs. 7.461 ± 0.918 kgr, $p=0.6$; Table 3.6). The accelerated growth noted was driven by FM accumulation, as GDM group continued to have lower FFM (Figure 3.6).

After adjusting for infant size using FMI, a mixed 3 x 2 multi-factorial ANOVA revealed a significant interaction between GDM and FMI, $F(2, 387) = 5.235$, $p=0.01$. Further examination with three independent “t-tests” and after adjusting for significance revealed that GDM group had higher FMI at visit 4 ($p=0.015$). FMI remained higher in the GDM group even after adjusting for maternal characteristics (Table 3.7).

Visit 4 (5 months)	GDM (n=42)	nGDM (n=150)	P VALUE
Age (days)	158 (20)	159 (13)	0.5
Weight (kgr)	7.432 (0.825)	7.461 (0.918)	0.6
Length (m)	0.65 (0.02)	0.65 (0.02)	0.73
%FM	26.7 (5.7)	25.8 (5.6)	0.6
FM (kgr)	2.003 (0.554)	1.945 (0.566)	0.9
FFM (kgr)	5.428 (0.636)	5.515 (0.644)	0.8
FMI (kgr/m ²)	5.1 (1.9)	4.5 (1.3)	0.015
FFMI (kgr/m ²)	12.7 (1.2)	12.8 (1.3)	0.7
Weight gain Visits ₂₋₄	4.312 (0.836)	4.138 (0.831)	0.2
Weight gain Visits ₃₋₄	3.226 (0.694)	3.078 (0.670)	0.2
Feeding mode	n (%)	n (%)	
Breastfeeding	11 (26.1)	34 (22.6)	0.1
Formula feeding	31 (73.8)	103 (68.6)	
Mixed	0(0)	13 (8.6)	
Breastfeeding duration (weeks)*	5.5 (0-20)	3.5 (0-20)	0.7

Table 3.6: Infant characteristics and feeding mode at visit 4. GDM infants develop an early adiposity rebound, still having lower FFM.

*Results expressed in medians and inter-quartile range

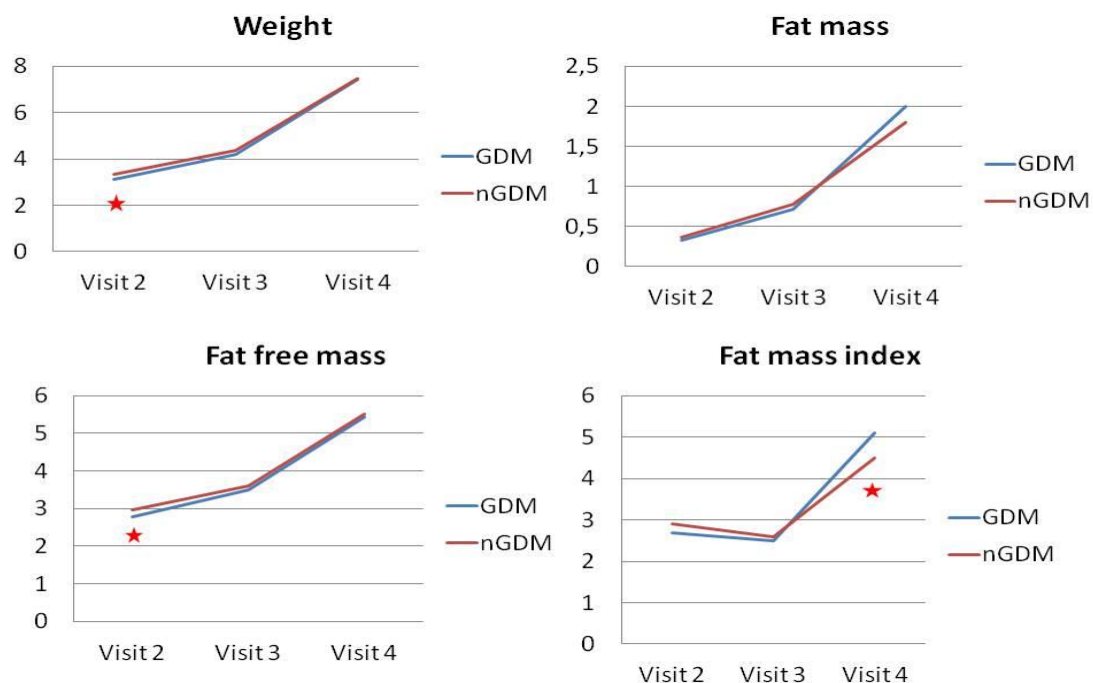


Figure 3.6: Weight and body composition distribution across the three visits (V2: birth, V3: 4 weeks, V4: 5 months). GDM offspring have an early adiposity rebound at 5 months; GDM (n=42), nGDM (n=150); Asterisk indicates statistically significant difference

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
1 (Constant)	4.758	1.821		2.612	0.01
GDM	0.639	0.288	0.175	2.216	0.02
BMI	0.010	0.018	0.045	0.559	0.57
GWG	0.002	0.017	0.010	0.118	0.90
GA	-0.013	0.045	-0.022	-0.292	0.77

a. Dependent Variable: **FMI at visit 4 (5 months postpartum)**

Table 3.7: Regression analysis of fat mass index (FMI) at visit 4 (5 months) adjusting for maternal BMI, maternal gestational weight gain (GWG) and gestational age (GA). GDM is an independent factor for offspring FMI at 5 months

3.2.6 Discussion

Results of our study revealed three key findings:

1. Treatment of GDM resulted in lower birthweight, which was driven by lower FFM, whereas FM was similar between groups;
2. Timing of delivery and gestational weight gain are independent predictors of FFM in the GDM group and
3. Offspring of GDM pregnancies, despite optimal maternal glycaemic control (indicated by HbA1c), have greater adiposity at 5 months.

To our knowledge, this is the first study to assess the evolution of adiposity in cases of treated GDM up to 5 months post partum. We have used fetal scan data in an attempt to track the origins of the disease from the antenatal period and we adjusted all our body composition measurements for infant size by using FMI and FFMI in order to provide statistically robust data.

Sonographic estimation of AC and EFW during the third trimester has been shown to have a sensitivity of 80% in detecting neonatal macrosomia (52, 53). Recent data suggests that abnormal fetal growth is present even before the biochemical diagnosis of GDM, which is usually at 28 weeks of gestation following OGTT. Sovio et al (54) in a UK cohort (85% White Caucasians, 15% Asians and Africans) showed that GDM fetuses have higher AC at 28 weeks and an accelerated growth velocity between 20 and 28 weeks of gestation compared to nGDM. Venkataraman et al (55) using anterior

abdominal wall thickness as a marker of abdominal adiposity showed that adverse body composition is present from 20 weeks of gestation in GDM pregnancies of Asian origin. One of the latest studies assessing fetal growth in both European and Asian population suggested a biphasic trend in the fetal development of GDM cases (56). Specifically, fetuses tend to be smaller between 12-16 weeks of gestation but demonstrate higher EFW, HC and AC by 24 weeks. In our cohort data was available from the 20 weeks anomaly scan. Consistent with findings from Brand et al (56) fetuses of GDM pregnancies were found to have higher EFW and HC. AC, although numerically higher in GDM cases, was only approaching statistical significance. We assume that the pathophysiology underpinning the biphasic fetal growth in GDM pregnancies is similar to this in cases of pre-existing diabetes (57). Hyperglycemia leads to low grade oxidative stress and pro-inflammation which in turn causes inhibition of trophoblast and placental growth. This growth restriction up to 16 weeks of gestation is followed by an accelerated “catch up” growth due to the hyperinsulinaemic environment (58).

Diagnostic criteria and management of GDM are predominantly based on the findings of HAPO study (59). This landmark study revealed a linear association between maternal hyperglycaemia and adverse perinatal outcomes without proposing a clear diagnostic or therapeutic threshold. As a result, various diagnostic criteria and therapeutic targets have been proposed, as already described in chapter 1. Common denominator for all the criteria is the aim to minimize perinatal outcomes without compromising healthy fetal growth. NICE 2015 criteria on GDM were adopted in our study (49). Strong evidence suggests that treatment of GDM, as proposed by current guidelines, significantly reduces LGA, macrosomia and shoulder dystocia (60, 61). On the other hand, despite maternal antenatal treatment, offspring of GDM pregnancies remain at higher risk of childhood obesity (62, 63). The exact mechanism remains unclear and genetic, epigenetic, maternal and environmental factors have been suggested as possible explanations.

NICE guidelines on GDM suggest to allow pregnancies up to 41 weeks of gestation provided that fetal growth and maternal glucose control is stable (47). Diabetes during pregnancy increases the risk of stillbirth by 4-6 times. In 50% of the cases stillbirths are described as idiopathic but maternal obesity, high maternal age and macrosomia are known risk factors (64). Rosenstein et al (65) in a retrospective analysis of over a million pregnancies (193,000 GDM) showed that GDM pregnancies are at higher risk of stillbirth between 36-39 weeks, with a relative risk ranging from 1.45 to 1.84. Results suggested that the risk of expectant management compared to delivery in GDM cases is higher after 38 weeks (Figure 3.7). These results should be interpreted with caution as the GDM cohort was also found to have higher maternal age, higher rates of hypertension and pre-

eclampsia, all known risk factors for stillbirth. Furthermore, the incidence of stillbirth was extremely low, not exceeding 0.06% of cases. A RCT assessing the optimal delivery time for insulin treated GDM cases, suggested that induction of labor at 38 weeks reduces incidence of macrosomia and SD without increasing the CS rate (66, 67). RCOG adopts NICE guidelines but recommends to “consider personal circumstances” when deciding about the time of delivery.

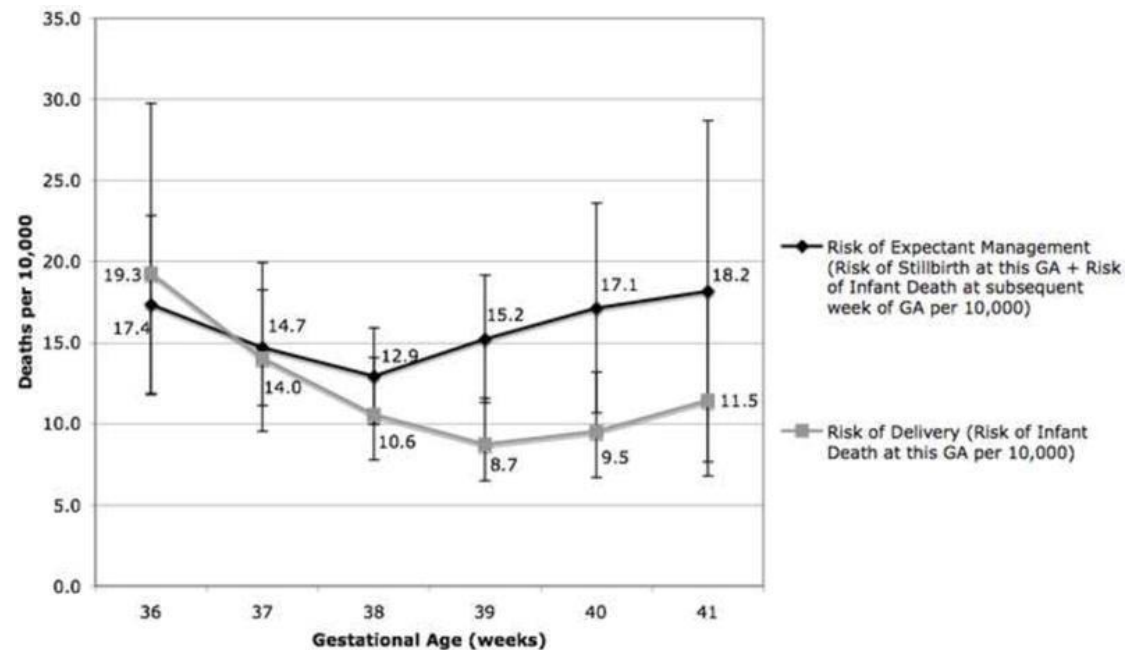


Figure 3.7: Mortality risk of expectant management versus induction of labour in GDM pregnancies. Risk increases significantly after 38 weeks gestation; Adopted from Rosenstein et al (65)

Our study compares a cohort of pregnant women without GDM receiving standard antenatal care with treated GDM pregnancies as per NICE guidelines. Maternal glycaemic control was optimal in the GDM group as indicated by a mean HbA1c of 34.9 mmol/mol. Intensified care of the GDM group with nutritional advice, close blood glucose monitoring, regular antenatal appointments resulted in lower gestational weight gain and earlier induction of labour. Offspring of GDM group were lighter with similar %FM with nGDM group but with significantly lower FFM. After adjusting for maternal characteristics and gestational age difference in FFM became nonsignificant indicating that the earlier induction and lower weight gain noted in the GDM group resulted in lower neonatal FFM. Gestational age had a higher effect size on FFM than weight gain pregnancy, as indicated by the standardized B co-efficient (GA: 0.378 vs. Wt gain: 0.185). The effect size of gestational age on FM and FFM in our cohort is consistent with findings from the INTERGROWTH 21st study (1). After analyzing a sample of 1,000 neonates using PEAPOD, the INTERGROWTH team showed that after 34 weeks of gestation FM

increases by a mean value of 33-36 gr/week and FFM by 155-169 gr/week (combined data for both sexes). In our study, GDM pregnancies were delivered 9 days earlier resulting in a mean FM difference of 52 gr and a mean FFM difference of 193 gr.

One of the most striking differences in the maternal characteristics of our cohort is the significantly less gestational weight gain noted in the GDM group. Regular antenatal appointments with dietician input, maternal education on healthy diet options and lifestyle changes implemented as part of the GDM management are more likely to be the cause for this difference. In 2009, IOM developed guidelines on optimal gestational weight gain based on prepregnancy maternal BMI (68). Purpose of the guidance was to balance the risks of having LGA infants, SGA infants, preterm births and postpartum weight retention. Publications of the guidelines triggered reactions, as many physicians claimed that targets are too high and contributing to obesity epidemics. Up to date results remain controversial, with some studies suggesting no harm in setting lower targets (69) and other linking weight gain below the recommendations with higher proportion of SGA, especially in obese and morbidly obese population (70). To our knowledge there is no study assessing the impact of weight gain below IOM recommendations on neonatal body composition using direct techniques. In our study, based on mean maternal booking BMI, GDM group had less weight gain than IOM targets (IOM based on BMI>30 kgr/m²: 5-9 kgr vs.GDM group: 3.8 kgr). We believe that in addition to earlier iatrogenic delivery, this significantly lower gestational weight gain has also contributed to the lower birthweight, with a more prominent effect, worryingly, on FFM.

By 5 months of age, we presented significant body composition differences between the two groups. GDM offsprings had higher weight gain which was driven by FM accumulation, as they continued to have lower FFM. We used statistically and clinically appropriate indices to adjust for infant size. Our analysis revealed that GDM group had higher adiposity at 5 months. Findings remained unchanged after adjusting for maternal characteristics, indicating a direct effect of GDM on infant body composition.

Our results are consistent with previous studies. Logan et al (71) assessing body composition with MRI in a UK cohort found no differences at birth between well controlled GDM pregnancies and nGDM. Similarly, Au et al (72) in an Australian cohort using PEAPOD and Brumbaugh et al (73) in US cohort using MRI failed to show any between group differences. We suggest that tight maternal glycaemic control, present in all studies, attenuated any differences in neonatal adiposity. Logan et al (71) after reassessing body composition at 10 weeks showed a 16% higher adiposity in the GDM group. Study group hypothesized that these differences may be due to altered

hypothalamic development and altered breast milk composition in the GDM group. Our study assessed infants at 4 weeks and 5 months post partum. There was no difference observed at 4 weeks but clearly, at 5 months, our GDM group demonstrated accelerated infant growth with excessive fat mass accumulation. We strongly believe that this altered growth is driven by the lower FFM at birth.

Our study is the first to look at FFM differences as the majority of literature focuses on the effect on FM. FFM is the most important factor of energy expenditure and accounts for 75% of Basic Metabolic Rate (BMR). Brain and liver are responsible for 58-70% of BMR whereas muscles account for 5% (Figure 3.8) (74). Dulloo et al (75) has nicely described the “active and passive” role of FFM on energy metabolism and body composition. Although appetite is predominantly adipocentric because of the close relationship between fat cells and leptin, FFM remains a physiological source of hunger (Figure 3.9). Increasing amount of FFM results in increased BMR which in turn leads to higher energy intake to match the requirements (passive role). On the other hand, loss of FFM triggers a feedback mechanism to increase intake in an attempt to restore FFM (active role; Figure 3.10). The Minnesota experiment (76) showed that human body in periods of starvation loses FM at greater rate compared to FFM. During refeeding though, FFM takes longer to restore leading to excessive weight gain and FM accumulation, a condition known as “collateral fattening”. In other words, despite FM and weight recovery hyperphagia persists until FFM is completely restored (Figure 3.11). In our cohort, we suggest that the reduced FFM noted at birth in the GDM group, leads to hyperphagia and weight gain in an attempt to restore FFM resulting in FM accumulation by 5 months of age. Rapid weight gain and higher FM are both independently linked to higher risk for future obesity (77).

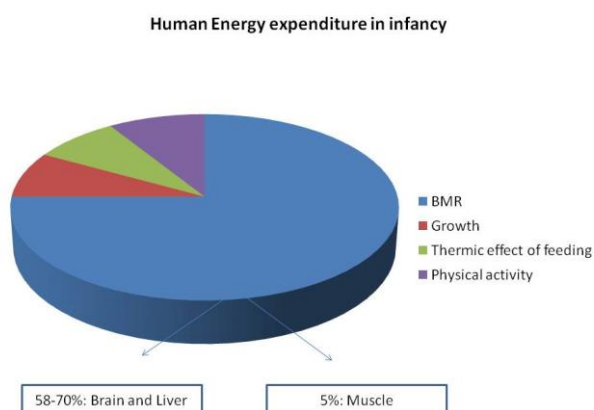


Figure 3.8: Energy expenditure in infancy; Basic Metabolic Rate (BMR) is the main contributor of total energy expenditure. Growth is the anabolic process requiring energy; Thermic effect of feeding is the energy required for food digestion and absorption

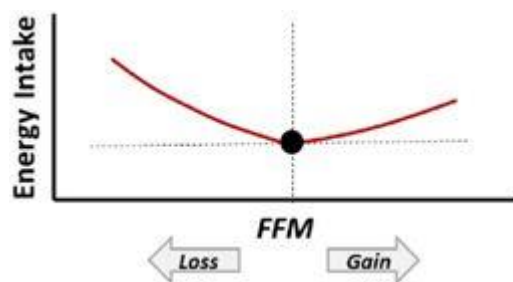


Figure 3.9: Energy intake and changes in fat free mass (FFM); Adopted from Dulloo et al (75)

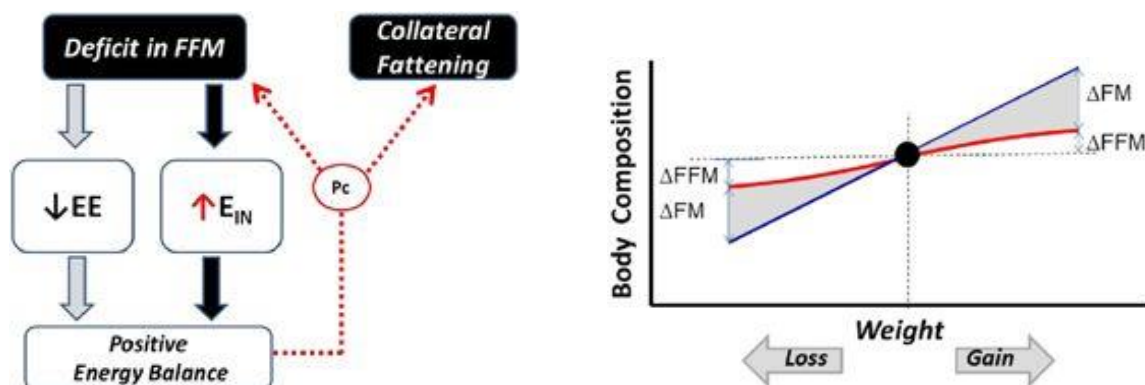


Figure 3.10: a) Active role of fat free mass (FFM; EE: energy expenditure, E_{IN} : energy intake), b) Impact of weight changes in body composition. Weight changes are predominantly driven by FM changes; Adopted from Dulloo et al (73)

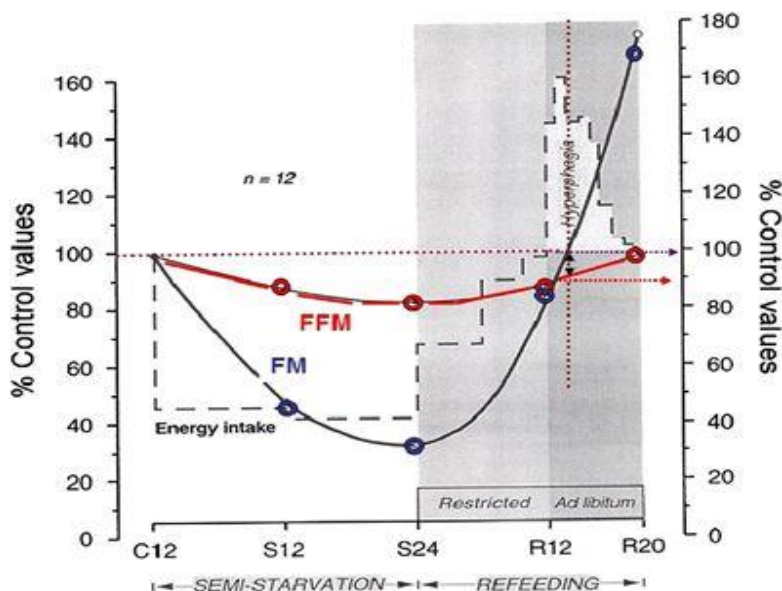


Figure 3.11: Changes in body composition during starvation and refeeding period (Minnesota experiment). During refeeding, hyperphagia persists, despite FM restoration, until FFM stores are depleted; Adopted from Dulloo et al (73)

One of the main limitations of our study is the lack of a healthy control group, a cohort which does not fulfill any of the high metabolic risk criteria. According to our study protocol, recruitment of low risk pregnancies could happen during booking visit, at 12 weeks of gestation. In real life though, recruitment of this low risk group proved to be challenging, as only two participants were recruited during early pregnancy. First trimester is a very sensitive period for mothers, with most of them not disclosing their pregnancy until the viability of the fetus is confirmed at booking visit. The above observation could potentially explain the small numbers expressing their wish to participate into research through their general practitioner or community midwife. Furthermore, low risk antenatal clinics tend to be larger and busier, making it difficult for private conversations between mothers and research staff. Potential participants may feel uncomfortable by the time limitations and the waiting room environment, thus deciding not to take part in the suggested study. Although our research staff always tried to respect privacy when talking to mothers about a very sensitive topic, the external impacts described before, seemed to have grossly affected their final decision. On the other hand, the comparison of the GDM group with a low risk group would have amplified the differences found in our study; therefore, we suggest that the lack of a healthy control group is not affecting the credibility of our results.

3.2.7 Conclusion

Our study is the first to follow the body composition evolution in pregnancies complicated by GDM up to 5 months of age. We showed that treatment of GDM results in a reduction of neonatal macrosomia at the cost of reduced FFM. This adverse phenotype at birth leads to rapid weight gain and FM accumulation by 5 months of age. The above indicate that maternal glycaemic control on its own may not be sufficient to prevent long term adverse outcomes and the correlation between GDM and childhood obesity is traced back in the antenatal and early post natal periods. The “gold standard” approach to pregnancies complicated by GDM, from preconception to the postnatal period, is yet to be identified and further studies are required to shed light into ways of preventing the adverse transgenerational effect of GDM.

3.2.8 References

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Chapter 4

Maternal adverse characteristics and ethnicity as predictors of infant body composition

4.1 Introduction

Prevalence of childhood obesity has reached an alarming rate constituting one of the most serious Public Health challenges globally (1). In chapter 1 we described the multifactorial origins of the disease and the higher risk of non-communicable diseases at a younger age. There is accumulating evidence that the origins of obesity can be traced as back as early postnatal life, antenatal and even prenatal health of the mother (2). Events occurring during this “plastic period” of development have multigenerational impact on human metabolism (Figure 4.1).

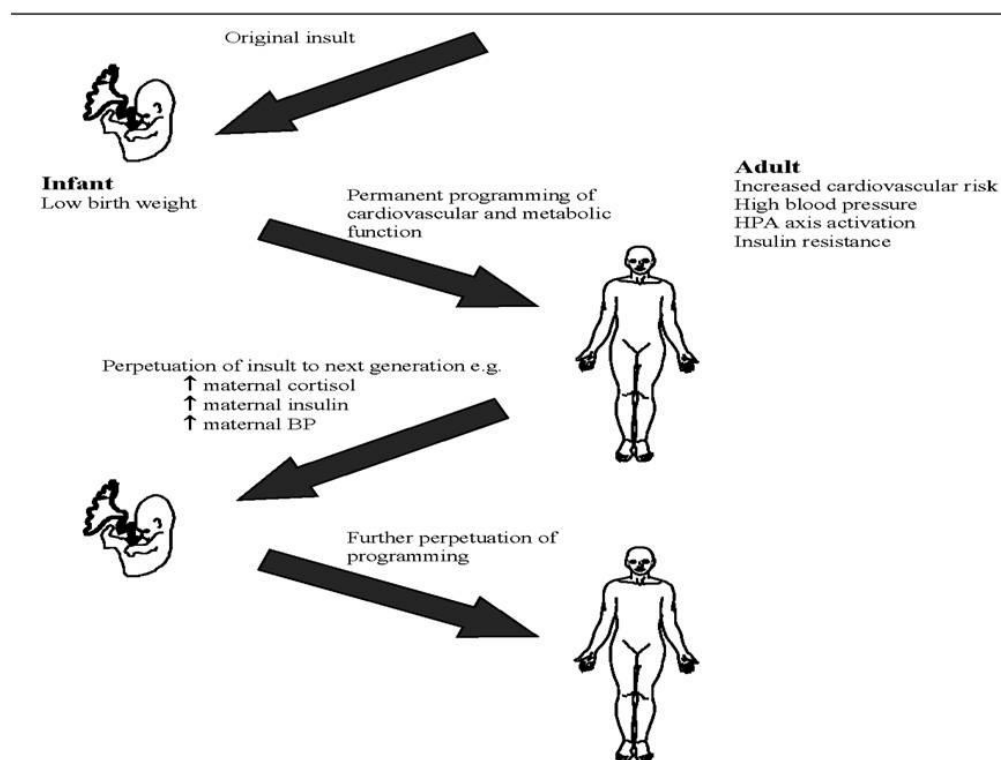


Figure 4.1: Intergenerational programming. The metabolic syndrome trait passes from generation to generation Adopted from Drake et al (3)

4.1.1 Risk factors

4.1.1.1 Maternal Obesity and gestational weight gain (GWG)

Maternal BMI has been considered one of the strongest predictors of offspring obesity in childhood and adulthood (4, 5). Neonatal body composition is closely linked to maternal phenotype, as mothers with increased adiposity are more likely to have infants with

excessive fat mass (FM) (6). GWG has less effect, compared to BMI, on adverse metabolic profile (7). Although the majority of studies reveal an association between excessive weight gain during pregnancy and childhood obesity (8-10), few failed to show any link with offspring body composition and BMI (11, 12).

4.1.1.2 Gender and ethnic differences

Gender and ethnic inequities in obesity are well described. Females are known to have higher %FM and lower FFM for a given BMI compared to males and are characterized by the “pear shaped” phenotype with increased subcutaneous fat deposition around the pelvic area. On the other hand, males despite having lower %FM are at higher risk of cardiometabolic diseases, as their fat is predominantly centrally distributed (“apple shaped”) (13). Sex specific genetic variants and sex hormones (estrogens, testosterone) are the main determinants of this gender dimorphism (14).

The Asian population is known to have a more adipose body composition for a given weight with increased central and visceral fat deposition (15). Evidence suggests that this adverse phenotype is present at birth (16). Countries of the South Asian (SA) continent are currently facing the “double burden” of the obesity epidemic: The problem of malnutrition is still present, especially in rural areas, but at the same time, in urban settings, risk factors of obesity and non-communicable diseases continue to rise. However, in the last decade a gradual “urbanization” of rural areas is noted. As such, fetal and early post natal periods, characterized by inadequate nutrition, are followed by exposure to high fat and energy dense foods as children grow older. This diet pattern coupled with lack of physical activity and sedentary lifestyle leads to an adipose phenotype and increased cardiometabolic diseases. Of great interest, studies taking place during the first decade of our century revealed that the “thin fat” phenotype is evident also in SA migrants to high-income countries, but differences from populations native to the high-income countries are attenuated when compared to residents of the SA continent (17).

4.1.1.3 Feeding patterns

BF during infancy is shown to reduce childhood obesity as well as having many other benefits for child health and wellbeing (18). The global recommendation is exclusive breast feeding for the first 6 months of life. Despite the beneficial effects of BF, only 30% of UK born infants are exclusively breastfed at 6-8 weeks postpartum (19). Recent

evidence suggests that exclusive BF beyond five months reduces the risk of obesity at 15 years of age in children with increased genetic risk (FTO gene) (20). Data on the effect of BF on body composition during the neonatal period remain scarce. A single US cohort study revealed that breastfed infants gained less weight than formula-fed infants up to 7 months, but interestingly this difference was driven by lower FFM accumulation, measured by DXA (21). At the same time, evidence from small cohorts reveal that breastfed infants have lower FFM and higher %FM during the first months of life, with the exact mechanism remaining unknown (22, 23).

4.1.2 Hypothesis

Adverse maternal characteristics, such as obesity and increased gestational weight gain, are linked to increased offspring adiposity in childhood. Similarly, Asian populations and females are known to have higher percentage of fat mass for a given weight when compared to those of white European descent and males respectively. We hypothesize that this altered body composition is present at birth and early infancy.

4.1.3 Research questions

- Is maternal adverse metabolic profile linked to altered body composition at birth and up to 5 months of age?
- Is the “thin-fat” phenotype present in infants of South Asian origin born in UK?
- Is there any difference in body composition between breastfed and formula-fed infants?
- Is gender dimorphism in body composition present during early infancy?

4.1.4 Outcomes

- Assess whether higher maternal BMI and gestational weight gain are linked to increased infant adiposity
- Assess ethnic-specific body composition variability
- Assess body composition differences between breastfed and formula-fed infants
- Assess total adiposity and adipose tissue distribution between male and female infants

4.1.5 Criteria

The current analysis includes participants of the longitudinal, cohort study assessing body composition differences between offspring of GDM and non GDM mothers, described in chapter 3. For this reason inclusion and exclusion criteria remain the same as previously described.

4.1.6 Methods – Statistical analysis

In order to assess the impact of maternal and infant characteristics on offspring body composition without the confounding effect of GDM treatment, we included data obtained only from the control group (ie. participants without GDM; n=211, Figure 4.2). We assessed the effect of maternal characteristics on infant body composition at three different time points, using data obtained from PEAPOD measurements. In order to assess the effect of intrauterine exposures on body composition at birth, data from all newborns (except cases of GDM) measured at birth was considered. The effect of maternal characteristics on the evolution of body composition during infancy was assessed using data from infants who completed all three study visits (n=150). In an attempt to detect differences originating from early fetal life we assessed fetal size data from the 20 weeks anomaly scan. Finally we used anthropometric measurements, in specific skinfold thickness, in order to determine differences in fat distribution. The ratio of sub scapular to triceps (SS/TR) skinfold thickness was used as a marker of central adiposity whereas their sum (SS+TR) as a marker of total adiposity (24).

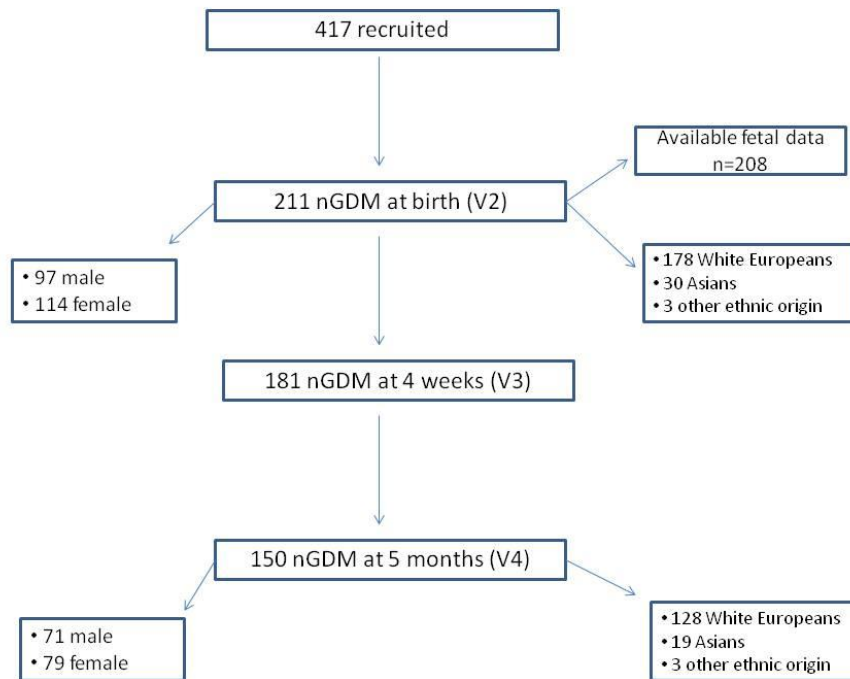


Figure 4.2: Flowchart detailing infant attendance per study visit. 150 nGDM infants were included for final analysis

We used multiple linear regression analysis and Generalized Linear Models (GLM) to assess the effect of maternal characteristics on body composition at birth and over a period of five months respectively. GLM can analyze the simultaneous effects of multiple variables, including mixtures of categorical and continuous variables. Ordinary linear regression assumes that a constant change in a predictor leads to a constant change in the response variable. We know that early postnatal life is a developmental period sensitive to environmental characteristics during which body composition undergoes dynamic changes (25), therefore GLM was used to describe the patterns of interactions and associations (26).

We assessed the effect of ethnicity, gender and feeding mode on body composition by separate regression models. Maternal and infant characteristics and offspring body composition were described separately according to the variable studied. Depending on feeding mode during the first 5 months participants were categorized into predominantly breastfeeding (BF), predominantly formula feeding (FF) and mixed feeding (similar proportions of breast and formula milk). Maternal ethnicity was determined using questionnaires which asked participants to indicate which ethnic category best described their ethnic origin. Women were classified as either White Europeans or South Asians (SA). The first group included mothers who were White British or of other White European descent and the latter group included participants originating from Bangladesh,

Nepal, India, Pakistan and Sri Lanka. We restricted the cohort to women of White European and SA origin, as numbers from other ethnic backgrounds were not adequate to be analyzed separately (n=3). We used the terms “first generation migrant” to describe a native born participant (mother) whose parents were born in another country and “second generation” if both participant and her parents were native born citizens. The ethnicity of the participant’s partner and grandparents was not captured in our dataset.

Statistical analysis was performed using “t test” or “Mann-Whitney U test” depending on data distribution (parametric or not). Independent one-way and mixed multi-factorial ANOVA was used to compare data across three or more independent variables. Chi-squared test was used to compare categorical data. For multivariable analysis, linear regression models were applied to adjust for maternal and infant characteristics. %FM was used to adjust absolute number of fat mass for infant weight whereas optimal indices (FMI, FFMI) were used to adjust for infant size.

Statistical analysis was performed using SPSS version 22. The accuracy of the methods and results were reviewed by the Chief Investigator’s institution.

4.2 Results

4.2.1 Predictors of neonatal body composition

Data from 208 participants was available and considered for the final analysis (Table 4.1). Maternal BMI, GWG, gestational age, ethnicity and gender were the variables examined. Maternal, fetal and neonatal characteristics were similar between the group who completed only visit 2 and those who completed all study visits.

n =208	Mean values (SD)
Maternal BMI (kgr/m²)	29.5 (6.9)
GWG (kgr)	8.1 (3.6)
Gestational age (wks)	39.3 (2.4)
Gender	Male: 94, Female: 114
Maternal Ethnicity	White: 178, Asian: 30

Table 4.1: Maternal characteristics. Our study cohort included infants of different ethnic background and gender

4.2.1.1 At birth

Maternal BMI, GWG and gestational age independently predicted birthweight. Maternal BMI was the strongest predictor of FM at birth; GWG, gestational age and gender also significantly contributed to the model examined (BW, FM and FFM). Gestational age, GWG, gender but not BMI predicted FFM at birth. Females were found to have higher FM and lower FFM at birth. Mother's ethnicity was not a predictor of any of the outcomes examined. Detailed effect size is shown in Table 4.2.

4.2.1.2 During the first 5 months of life

Maternal BMI and GWG were the only variables which independently predicted all outcomes (weight, FM, FFM). Gestational age was linked to weight and FFM, whereas the association with FM was approaching statistical significance ($p=0.08$). Male sex was related to higher weight and FFM. Ethnicity and breastfeeding duration were not found to predict body composition during the first five months post partum (Table 4.3, Figure 4.3).

a)

Birthweight (n=208)	β-coefficient	Standardized B
Maternal BMI (kgr/m²)	0.012*	0.162
GWG (kgr)	0.011*	0.194
Gestational age (wks)	0.077*	0.384
Gender	-0.115	-0.111
Ethnicity	-0.178	-0.121

b)

FM at birth (n=208)	β-coefficient	Standardized B
Maternal BMI (kgr/m²)	0.006*	0.250
GWG (kgr)	0.004*	0.196
Gestational age (wks)	0.014*	0.219
Gender	0.059*	0.175
Ethnicity	-0.063	-0.131

c)

FFM at birth (n=208)	β-coefficient	Standardized B
Maternal BMI (kgr/m²)	0.006	0.107
GWG (kgr)	0.007*	0.167
Gestational age (wks)	0.063*	0.397
Gender	-0.172*	-0.210
Ethnicity	-0.118	-0.102

Table 4.2: Results of multiple regression analysis showing predictors of body composition (a: birthweight; b: FM; c: FFM) at birth. Gestational age and weight gain independently predicted all studied outcomes, whereas BMI only birthweight and FM; * p value <0.05

n=147	Weight (kgr)	FM (kgr)	FFM (kgr)
Maternal BMI	0.021*	0.009*	0.011*
GWG (kgr)	0.017*	0.006*	0.012*
Gestational age (wks)	0.069*	0.01	0.058*
Gender	0.52*	- 0.06	0.583*
Ethnicity	0.07	- 0.02	0.107
Breastfeeding duration (wks)	0.006	0.004	0.002

Table 4.3: Predictors of body composition during the first 5 months, values represent β co-efficient. Maternal BMI and gestational weight gain independently predicted weight, FM and FFM during this period; * p value <0.05

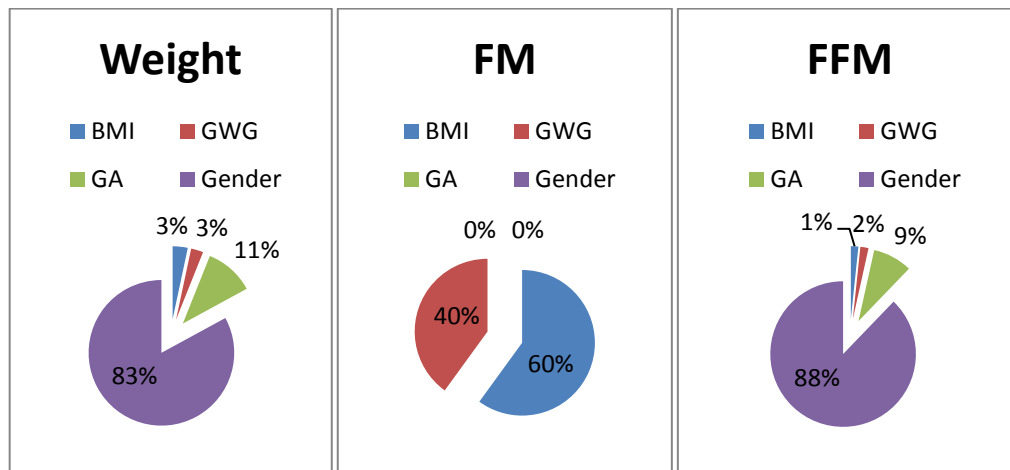


Figure 4.3: Predictors of weight, fat mass (FM) and fat free mass (FFM) during the first 5 months of life; BMI: maternal booking BMI; GWG: gestational weight gain; GA: gestational age

4.2.2 Body composition and Gender

Maternal characteristics and ethnic variation were similar between the two groups. Despite a similar weight at birth, females were found to have higher %FM ($11.6 \pm 4.3\%$ vs. $9.5 \pm 3.8\%$, $p < 0.01$), higher FM (0.40 ± 0.171 vs. 0.334 ± 0.163 kgr, $p < 0.01$) and FMI (3.2 ± 1.3 vs. 2.5 ± 1.1 kgr/m³, $p < 0.01$) but lower FFM (2.886 ± 0.373 vs. 3.043 ± 0.421 kgr, $p < 0.01$). Body composition differences attenuated and became non significant at 4 weeks (Table 4.4).

a)

	Male (n=94)	Female (n=114)	p value
Maternal Age (years)	33.6 (5.4)	31.2 (5.1)	0.2
Maternal height (m)	1.64 (0.06)	1.65 (0.07)	0.4
Ethnicity	White European: 81 Asian: 13	White European: 97 Asian: 17	0.9
BMI (kgr/m²)	29.9 (6.7)	29.2 (7.0)	0.5
GWG (kgr)	8.454 (4.891)	6.295 (4.358)	0.09
Gestational Age (wks)	39.2 (3.3)	39.4 (1.3)	0.6

b)

Visit 2 (birth)	Male(n=94)	Female(n=114)	p value
Birthweight (kgr)	3.374 (0.522)	3.284 (0.499)	0.2
Length (m)	0.5 (0.02)	0.49 (0.02)	0.1
%FM	9.5 (3.8)	11.6 (4.3)	<0.01
FM (kgr)	0.334 (0.163)	0.400 (0.171)	<0.01
FFM (kgr)	3.043 (0.421)	2.886 (0.373)	<0.01
FMI (kgr/m³)	2.5 (1.1)	3.2 (1.3)	<0.01
FFMI (kgr/m³)	23.9 (2.8)	24.0 (3.0)	0.5

c)

Visit 3 (4-5 weeks)	Male(n=68)	Female(n=79)	p value
Age (Days)	33.4 (6.5)	33.0 (5.7)	0.6
Weight (kgr)	4.470 (0.670)	4.321 (0.653)	0.1
Length (m)	0.54 (0.03)	0.54 (0.02)	0.06
%FM	17.0 (4.3)	18.2 (5.0)	0.1
FM (kgr)	0.776 (0.262)	0.798 (0.307)	0.6
FFM (kgr)	3.694 (0.484)	3.523 (0.452)	0.02
FMI (kgr/m²)	2.5 (0.7)	2.7 (0.9)	0.2
FFMI (kgr/m²)	12.2 (1.0)	12.0 (1.1)	0.4
Feeding mode	n (%)	n (%)	0.07
Breastfeeding	21 (31)	32 (41)	
Formula-feeding	31 (45.5)	39 (49)	
Mixed	16 (23.5)	8 (10)	

Table 4.4: a) Maternal characteristics b) Neonatal characteristics at birth c) Infant characteristics and feeding mode at visit 3 (4 weeks). Maternal characteristics were similar between groups. Female neonates were found to have higher FM but lower FFM

Weight gain (males: 4.225 ± 0.776 kgr, females: 4.028 ± 0.863 kgr; $p=0.1$) and age (males: 159 ± 12.9 days, females: 160 ± 14.8 days; $p=0.6$) at visit 4 (5 months) were similar between the two groups, thus male infants remained numerically heavier (7.595 ± 0.826 vs. 7.323 ± 0.980 kgr, $p=0.7$). Mixed 3 x 2 multi-factorial ANOVA revealed a significant interaction between gender and %FM [$F(1, 144)=14.724$, $p<0.01$], FFM [$F(1, 144)=12.738$, $p<0.01$], FMI [$F(1, 144)=10.897$, $p<0.01$] and FFMI [$F(1, 144)=2.988$, $p=0.05$]. Further examination with three independent “t-tests” and after adjusting for significance ($p<0.016$) revealed that %FM ($p<0.01$), FMI ($p=0.011$) and FFM ($p<0.01$) remained significantly different between groups (Table 4.5, Figure 4.4).

Visit 4 (5 months)	Male(n=68)	Female(n=79)	p value
Age (days)	159 (12.9)	160 (14.8)	0.6
Weight (kgr)	7.595 (0.826)	7.323 (0.980)	0.7
Length (m)	0.66 (0.02)	0.65 (0.02)	0.02
%FM	23.9 (4.8)	27.2 (5.7)	<0.01
FM (kgr)	1.834 (0.489)	2.025 (0.615)	0.04
FFM (kgr)	5.760 (0.592)	5.298 (0.600)	<0.01
FMI (kgr/m ²)	4.2 (1.1)	4.7 (1.3)	0.011
FFMI (kgr/m ²)	13.1 (1.0)	12.5 (1.3)	0.08
Feeding mode	n (%)	n (%)	0.2
Breastfeeding	13 (19)	20 (25)	
Formula feeding	47 (69)	55 (70)	
Mixed	8 (12)	4 (5)	
Breastfeeding duration (weeks)*	4 (0-20)	4.2 (0-20)	0.8

Table 4.5: Infant characteristics and feeding mode at visit 4 (5 months). Female infants continued to show higher adiposity and lower FFM

*Results expressed in medians and inter-quartile range

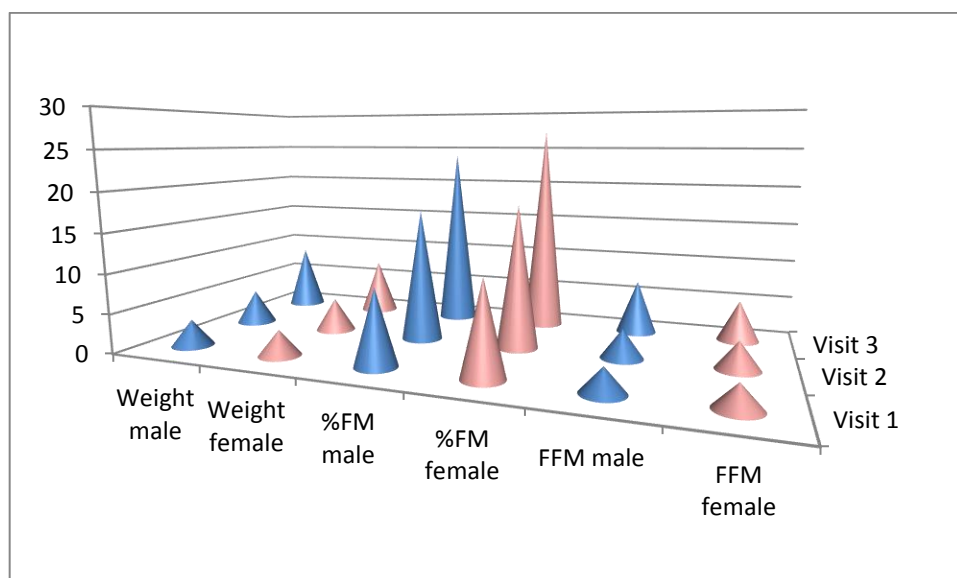


Figure 4.4: During the first 5 months of life female offspring had consistently lower weight, lower fat free mass (FFM) but higher percentage of fat mass (%FM)

4.2.2.1 Fetal and infant anthropometric measurements

Fetal scan at 20 weeks revealed that male fetuses had higher HC (177.6 ± 7.0 vs. 174.6 ± 7.5 mm, $p < 0.01$) and higher AC (157.1 ± 8.3 vs. 154.3 ± 8.1 , $p = 0.05$), which remained significant even after adjusting for maternal BMI, gestational age and ethnicity. Rest of the measurements were similar between the 2 groups (Table 4.6).

	Fetal scan		
	Male (n=94)	Female (n=114)	p value
Gestational age (weeks)	20.3 (0.4)	20.2 (0.4)	0.6
EFW (grams)	359.0 (39.1)	352.9(38.2)	0.2
HC (mm)	177.6 (7.0)	174.6 (7.5)	<0.01
AC (mm)	157.1 (8.3)	154.3 (8.1)	0.05
FL (mm)	32.7 (1.9)	33.0 (2.0)	0.2

Table 4.6: Fetal data during 20 weeks scan. Female fetuses were found to have lower head and abdominal circumference

Male neonates continued to have higher HC at birth. The remaining anthropometric measurements, including TR and SS skinfold thickness as well as waist (WC) – chest (CC) – arm (ARC) circumference were similar in the two groups. Consistent with the

PEAPOD measurements, females were found to have numerically, but not statistically, higher total adiposity without any differences in the distribution of adipose tissue during the follow up visits (Table 4.7).

At birth	Male (n=94)	Female (n=114)	Model 1 p VALUE	Model 2 p value
HC (cm)	35 (1.5)	34.2 (1.3)	<0.01	<0.01
TR (mm)	6.9 (3.9)	7.1 (1.9)	NS	NS
SS (mm)	6.9 (3.4)	6.9 (1.7)	NS	NS
SS/TR	1.03 (0.02)	0.98 (0.01)	NS	NS
SS+TR (mm)	13.9 (7.2)	14 (3.5)	NS	NS
WC (cm)	33.6 (2.8)	34.9 (2.1)	NS	NS
CC (cm)	34.6 (2.3)	34.9 (2.1)	NS	NS
ARC (cm)	13.1 (1.2)	11.2 (1.3)	NS	NS
Visit 3 (4-5 weeks)	(n=68)	(n=79)		
TR (mm)	8.4 (1.9)	9.2 (2.2)	<0.01	0.02
SS (mm)	8.3 (1.8)	8.9 (1.8)	<0.01	0.02
SS/TR	1.00 (0.17)	0.99 (0.16)	NS	NS
SS+TR (mm)	16.8 (3.5)	18.1 (3.8)	0.2	0.002
WC (cm)	38.1 (2.8)	37.8 (3.1)	NS	NS
CC (cm)	38.7 (2.6)	38.0 (2.6)	NS	NS
ARC (cm)	12 (1.3)	11.8 (1.3)	NS	NS
Visit 4 (4-5 months)	(n=68)	(n=79)		
TR (mm)	12.1 (3.1)	11.9 (3.2)	NS	NS
SS (mm)	9.80 (3.3)	10.80 (2.9)	NS	NS
SS/TR	0.82 (0.24)	0.90 (0.28)	NS	NS
SS+TR (mm)	21.9 (5.9)	22.8 (5.6)	NS	NS
WC (cm)	44 (3.1)	43.7 (3.4)	NS	NS
CC (cm)	44.9 (2.4)	44.0 (2.9)	NS	NS
ARC (cm)	14.9 (1.4)	14.7 (1.5)	NS	NS

Table 4.7: Infant anthropometric measurements based on gender, Anthropometric indexes did not reveal any sustained difference between groups TR: triceps skinfold thickness; SS: sub scapular skinfold thickness; WC: waist circumference; CC: chest circumference; ARM: arm circumference; NS : non significant ($p>0.05$); Model 1: unadjusted , Model 2 : adjusted for infant weight or length

4.2.3 Body composition and ethnicity

Maternal characteristics of the two groups can be found in Table 4.8. Both groups shared similar maternal and gestational age but SA group had lower BMI (30.3 ± 6.9 vs. 24.7 ± 5.3 kgr/m², $p < 0.01$) and higher weight gain during gestation (7.931 ± 4.312 vs. 9.270 ± 3.643 , $p = 0.5$). There was no difference in maternal total adiposity (SS+TR) or adipose tissue distribution (SS/TR) as indicated by the skinfold thickness, even after adjusting for maternal BMI.

	White European (n=178)	SA (n=30)	p value
Maternal Age (years)	32.4 (5.5)	31.5 (4.5)	0.8
Maternal height (m)	1.65 (0.06)	1.65 (0.08)	0.9
BMI (kgr/m²)	30.3 (6.9)	24.7 (5.3)	<0.01
GWG (kgr)	7.931 (4.312)	9.270 (3.643)	0.5
Gestational Age (weeks)	39.3 (2.6)	39.3 (1.2)	0.7
SS+TR (mm)	53.6 (13)	48.1 (11)	0.07
SS/TR (mm)	1.07 (0.2)	1.10 (0.2)	0.6

Table 4.8: Maternal characteristics between White Europeans and South Asians (SA). SA mothers had lower BMI but similar measures of adiposity

Socioeconomic data and diet patterns were retrieved from the study questionnaires. SA participants had higher educational qualifications and the vast majority was married. There were no between group differences in employment status, annual income and diet patterns (Table 4.9).

Socioeconomic		White European (n=178)	SA (n=30)	p value
Education (MD,PhD,PGCE)	Higher	51 (28.5%)	17 (56.5%)	<0.01
	Professional	25 (14%)	4 (13.5%)	
	Entry level	92 (52%)	6 (20%)	
	No qualifications	10 (5.5%)	3 (10%)	
Marital status	Married	100 (56%)	28 (93%)	<0.01
	Single	78 (44%)	2 (7%)	
Employment	Yes	138 (77%)	21 (70%)	0.8
	No	40 (23%)	9 (30%)	
Income	>52,000	45 (25%)	6 (20%)	0.09
	36,400-51,999	39 (22%)	3 (10%)	
	26,000-36,339	31 (17.5%)	5 (16.5%)	
	<26,000	63 (35.5%)	16 (53.5%)	
Diet	Vegetarian	7 (5.4%)	3 (10%)	0.6
	Non vegetarian	171 (96%)	27 (95%)	
Generation	First	NA	13	NA
	Second	NA	0	
	Immigrants	NA	17	

Table 4.9: Socioeconomic and diet characteristics of White European and South Asian (SA) study population. Of note the high percentage of higher education and marital status in the SA group; NA: Not Applicable, NR: Not Reported; p values represent statistical significance when compared to the SA population

4.2.3.1 Body composition at birth

There was no difference in gender between the two groups. Neonates of SA group were found to have a lower birthweight (3.368 ± 0.501 vs. 3.065 ± 0.497 kgr, $p<0.01$) which was driven by lower %FM (11.0 ± 4.0 vs. $8.7 \pm 4.0\%$, $p<0.01$), as FFM was similar between the two groups (2.985 ± 0.396 vs. 2.830 ± 0.441 kgr, $p=0.1$). Difference in FM remained after adjusting for neonatal length as there was a statistically significant difference in FMI between the two groups (White Europeans: 3.0 ± 1.2 kgr/m³, SA: 2.0 ± 1.1 kgr/m³, $p<0.01$). After applying multiple regression models adjusting for maternal BMI, educational and marital status the correlation between FM, %FM, FMI and ethnicity slightly attenuated but remained significant whereas differences in birthweight were no longer apparent (Table 4.10).

a)

Visit 2 (Birth)	White Europeans (n=178)	SA (n=30)	p value
Gender	Male: 82 Female: 96	Male: 13 Female: 17	0.9
Birthweight (kgr)	3.368 (0.501)	3.065 (0.497)	<0.01
Length (m)	0.5 (0.02)	0.49 (0.02)	0.4
%FM1	11.0 (4.0)	8.7 (4.0)	<0.01
FM (kgr)	0.385 (0.170)	0.276 (0.145)	<0.01
FFM (kgr)	2.985 (0.396)	2.830 (0.441)	0.1
FMI (kgr/m ³)	3.0 (1.2)	2.3 (1.1)	<0.01
FFMI (kgr/m ³)	23.8 (2.9)	23.2 (2.7)	0.4

b)

Visit 2 (Birth)	Model 1		Model 2	
	B coef.	Sig.	B coef.	Sig.
Birthweight (kgr)	-0.303	<0.01	-.251	NS
%FM	-2.36	<0.01	-1.83	0.02
FM (kgr)	-0.109	<0.01	-0.083	0.01
FMI (kgr/m ³)	-0.7	<0.01	-0.5	0.02
FFM (kgr)	-0.155	0.1	-0.132	NS
FFMI (kgr/m ³)	-0.6	0.4	-0.5	NS

Table 4.10: a) Neonatal characteristics at birth b) Regression of neonatal body composition between the two groups; SA infants presented lower weight and adiposity at birth, even after adjusting for maternal characteristics; Model 1: unadjusted, Model 2: adjusted for maternal BMI, marital and educational status, NS: Not Significant

4.2.3.2 Body composition at 4-5 weeks and 5 months

Maternal, fetal and neonatal characteristics were similar between the group who completed only visit 2 (birth) and those who completed all study visits. SA infants demonstrated numerically higher weight gain during the first 5 months of life (4.095 ± 0.850 vs. 4.272 ± 0.654 kgr, $p=0.3$) but still remained lighter than the White European cohort. Of great interest, the weight gain was predominantly driven by a more rapid accumulation of FM resulting in numerically, but not statistically, higher FM% at 5 months ($25.6 \pm 5.5\%$ vs, 26.6 ± 6.2 , $p=0.4$). FFM remained lower in the SA group throughout the

study. Results remained unchanged after adjusting for infant size (Figure 4.5, Table 4.11).

BF data revealed that SA mothers were more likely to breastfeed their infants postpartum, having a higher total median duration of BF (White European median: 3.0, IQR [0-20] weeks; SA median: 10.0, IQR [3.0- 20] weeks). After adjusting for maternal BMI, socioeconomic status and BF duration, body composition differences remained non significant between groups.

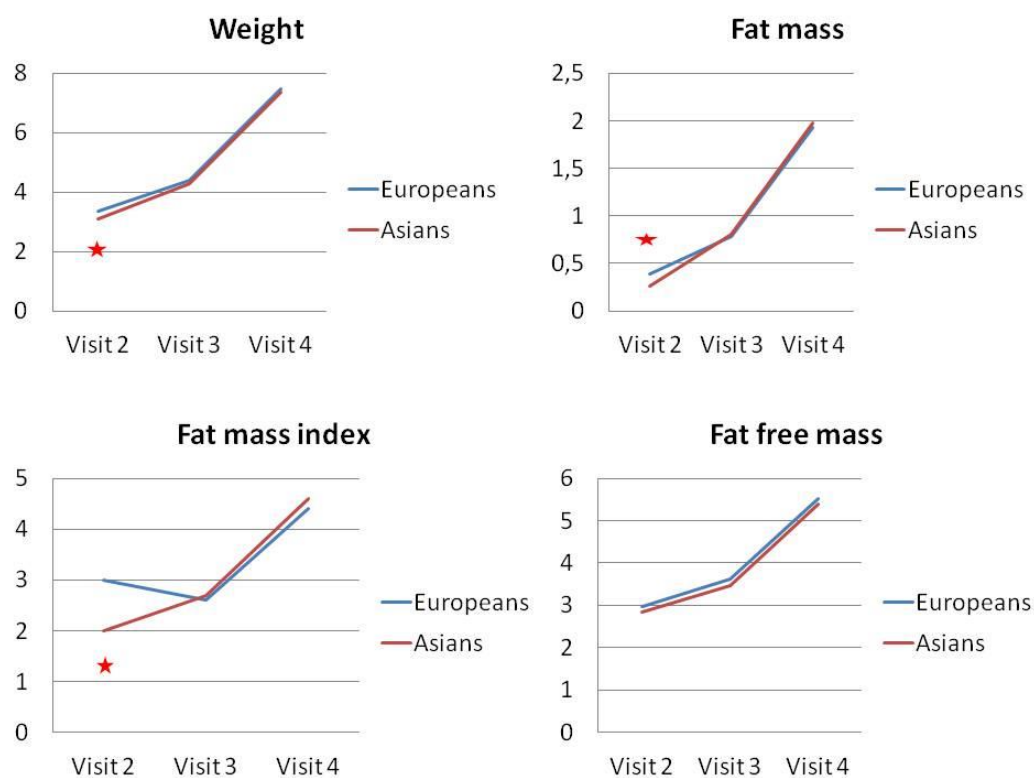


Figure 4.5: Body composition evolution between White Europeans and South Asians during the first 5 months of life. Both groups demonstrated similar body composition evolution after birth; Asterisk represents statistically significant difference

a)

Visit 3 (4-5 weeks)	White Europeans (n=128)	SA (n=19)	p value
Age (Days)	37.8 (6.0)	35.5 (6.3)	0.07
Weight (kgr)	4.405 (0.640)	4.290 (0.814)	0.4
Length (m)	0.54 (0.02)	0.54 (0.02)	0.6
%FM	17.5 (4.4)	18.2 (6.4)	0.5
FM (kgr)	0.784 (0.274)	0.811 (0.367)	0.7
FFM (kgr)	3.620 (0.460)	3.478 (0.554)	0.2
FMI (kgr/m ²)	2.6 (0.8)	2.7 (1.1)	0.7
FFMI (kgr/m ²)	12.2 (1.1)	11.7 (1.0)	0.09
Feeding mode	n (%)	n (%)	0.04
Breastfeeding	43 (33.5)	10 (52.5)	
Formula-feeding	66 (51.5)	4 (21)	
Mixed	19 (15)	5 (26.5)	

b)

Visit 4 (5 months)	White Europeans (n=128)	SA (n=19)	p value
Age (days)	160.5 (13.4)	153.5 (15.9)	0.04
Weight gain V2-V4 (kgr)	4.095 (0.850)	4.272 (0.654)	0.3
Weight (kgr)	7.462 (0.923)	7.368 (0.924)	0.6
Length (m)	0.65 (0.02)	0.65 (0.02)	0.5
%FM	25.6 (5.5)	26.6 (6.2)	0.4
FM (kgr)	1.931 (0.565)	1.981 (0.590)	0.7
FFM (kgr)	5.529 (0.629)	5.382 (0.693)	0.3
FMI (kgr/m ²)	4.4 (1.3)	4.6 (1.3)	0.6
FFMI (kgr/m ²)	12.8 (1.2)	12.6 (0.9)	0.4
Feeding mode	n (%)	n (%)	0.01
Breastfeeding	24 (19)	9 (47.5)	
Formula feeding	92 (72)	10 (52.5)	
Mixed	12 (9)	0	

Table 4.11: Infant characteristics and feeding mode between White Europeans and South Asians (SA) at a) 4 weeks b) 5 months. After adjusting for maternal characteristics and feeding mode ethnic background did not affect infant body composition

*Results expressed in medians and inter-quartile range

4.2.3.3 Fetal and infant anthropometric measurements

Fetal scan revealed that SA group had a lower estimated fetal weight at 20 weeks of gestation (348.3 ± 43.5 vs. 356.8 ± 37.8 grams, $p=0.3$) with similar HC and FL. AC was smaller in the SA group (154.8 ± 9.2 vs. 156.2 ± 8.8 mm, $p=0.4$). Results remained unchanged after adjusting for maternal BMI and height (Table 4.12).

	Fetal scan		
	White Europeans (n=178)	SA (n=30)	p value
Gestational age (weeks)	20.3 (0.4)	20.2 (0.6)	0.9
EFW (grams)	356.8 (37.8)	348.3 (43.5)	0.3
HC (mm)	176.0 (7.3)	176.2 (8.4)	0.9
AC (mm)	156.1 (8.8)	154.8 (9.2)	0.4
FL (mm)	32.8 (1.9)	33.1 (1.8)	0.7

Table 4.12: Fetal data during 20 weeks scan. Fetal size was similar between studied groups

After adjusting for infant's weight or length, neonatal anthropometry revealed a lower WC (32.2 ± 2.6 vs. 34.3 ± 2.6 cm, $p=0.02$) and lower SS/TR ratio (0.9 ± 0.1 vs. 1.02 ± 0.1 , $p=0.01$) in the SA group at birth, with the remaining measurements being similar between the two groups. There were no differences noted at visit 3. During visit 4, markers of total and central adiposity were similar between the groups and interestingly, SA were found to have higher arm circumference (15.5 ± 1.3 vs. 14.6 ± 1.5 cm, $p<0.01$; Table 4.13).

Visit 2 (birth)	White Europeans (n=178)	SA (n=30)	Unadjusted p VALUE	Adjusted for weight/length p value
HC (cm)	34.7 (1.5)	33.9 (1.5)	0.02	NS
TR (mm)	7.2 (3.1)	6.0 (1.3)	NS	NS
SS (mm)	7.1 (2.7)	5.5 (1.3)	0.01	NS
SS/TR	1.02 (0.1)	0.9 (0.1)	0.01	0.01
SS+TR (mm)	14.3 (5.7)	11.5 (2.4)	0.04	NS
WC (cm)	34.3 (2.6)	32.2 (2.6)	<0.01	0.02
CC (cm)	35.0 (2.2)	33.4 (2.1)	<0.01	NS
ARC (cm)	12.3 (8.7)	10.7 (1.1)	NS	NS
Visit 3 (4-5 weeks)	(n=128)	(n=19)		
TR (mm)	8.9 (2.1)	8.6 (2.0)	NS	NS
SS (mm)	8.7 (1.8)	8.4 (1.2)	NS	NS
SS/TR	0.99 (0.1)	0.99 (0.1)	NS	NS
SS+TR (mm)	17.6 (3.7)	17.1 (3.)	NS	NS
WC (cm)	38.0 (2.7)	37.4 (4.4)	NS	NS
CC (cm)	38.4 (2.5)	38.1 (3.5)	NS	NS
ARC (cm)	11.8 (1.3)	12.1 (1.4)	NS	NS
Visit 4 (5 months)	(n=128)	(n=19)		
TR (mm)	11.9 (3.2)	12.8 (2.4)	NS	NS
SS (mm)	10.1 (3.1)	11.6 (2.6)	NS	0.03
SS/TR	0.87 (0.2)	0.9 (0.1)	NS	NS
SS+TR (mm)	22.07 (5.8)	24.5 (4.9)	NS	NS
WC (cm)	43.9 (3.2)	43.7 (3.8)	NS	NS
CC (cm)	44.4 (2.7)	44.4 (3.3)	NS	NS
ARC (cm)	14.6 (1.5)	15.5 (1.3)	0.02	<0.01

Table 4.13: Infant anthropometric measurements based on ethnicity. Infant anthropometry did not reveal any sustained between groups difference; TR: triceps, SS: sub scapular, WC: waist circumference, CC: chest circumference, ARM: arm circumference; NS: non significant

4.2.4 Body composition and feeding mode

Data from 147 participants was reviewed and considered for final analysis. The majority of our participants were found to predominantly FF their offspring (n=102), followed by BF (n=33) and mixed feeding (n=12). Maternal characteristics were similar across the three groups. There was a trend for mothers who opted to formula feed their infants to be younger (BF: 33.5 ± 4.6, FF: 31.7 ± 5.4, Mixed: 31.3 ± 6.4 years; p=0.2) with higher BMI (BF: 28.3 ± 6.9, FF: 30.2 ± 7.1, Mixed: 29.9 ± 4.7 kg/m²; p=0.04; Table 4.14).

	Breast feeding (n=33)	Formula feeding (n=102)	Mixed (n=12)	p value
Maternal Age (years)	33.5 (4.6)	31.7 (5.4)	31.3 (6.4)	0.2
Maternal height (m)	1.63 (0.07)	1.64 (0.17)	1.62 (0.06)	0.9
BMI (kg/m²)	28.3 (6.9)	30.2 (7.1)	29.9 (4.7)	0.4
GWG (kg)	8.236 (6.414)	8.500 (8.192)	3.600 (5.063)	0.1
Gestational Age (weeks)	39.4 (1.4)	39.2 (3.2)	39.6 (1.0)	0.8
Ethnicity	White: 24 Asians: 9	White: 92 Asians: 10	White: 12 Asians: 0	0.6

Table 4.14: Maternal characteristics based on feeding mode

4.2.4.1 Body composition at birth and 5 months post partum (visit 4)

There was no significant gender dimorphism across the three groups. Neonatal characteristics were similar at birth. 36.5% of our participants were predominantly BF at 4 weeks and only 22.6% at 5 months. A mixed 2X3 multifactorial ANOVA revealed a significant interaction between FFMI [F(2,148)=4.149, p=0.01] at visit 4 and mode of feeding. BF infants had significantly lower FFMI from both FF and mixed fed infants (FFMI: 12.2 ± 0.9 vs. 12.9 ± 1.3 vs. 13.2 ± 1.7). Although not reaching statistical significance there was a clear trend for BF infants to gain less weight but have higher %FM and FMI (Table 4.15).

a)

Visit 2 (birth)	Breast feeding (n=33)	Formula feeding (n=102)	Mixed (n=12)	p value
Gender	Male: 13 Female: 20	Male: 47 Female: 55	Male: 8 Female: 4	0.8
Birthweight (kgr)	3.408 (0.604)	3.296 (0.506)	3.351 (0.358)	0.5
Length (m)	0.50 (0.02)	0.49 (0.02)	0.50 (0.01)	0.1
%FM1	10.8 (4.9)	10.7 (4.0)	9.2 (3.9)	0.4
FFM (kgr)	3.013 (0.450)	2.932(0.406)	3.024 (0.283)	0.5
FMI (kgr/m³)	2.9 (1.4)	2.9 (1.2)	2.5 (1.0)	0.6
FFMI (kgr/m³)	23.1 (2.4)	23.8 (3.1)	23.4 (2.9)	0.4

b)

Visit 4 (5 months)	Breast feeding (n=33)	Formula feeding (n=102)	Mixed (n=12)	p value
Weight (kgr)	7.419 (0.921)	7.451 (0.938)	7.652 (0.785)	0.7
Weight gain (kgr)	4.011 (0.886)	4.159 (0.827)	4.300 (0.731)	0.5
Length (m)	0.65 (0.03)	0.65 (0.04)	0.65 (0.02)	0.8
%FM1	27.7 (5.4)	25.1 (5.6)	25.8 (5.2)	0.06
FFM (kgr)	5.332 (0.588)	5.559 (0.678)	5.646 (0.419)	0.1
FMI (kgr/m³)	4.8 (1.2)	4.4 (1.3)	4.6 (1.4)	0.3
FFMI (kgr/m³)	12.2 (0.9)	12.9 (1.3)	13.2 (1.7)	0.01
Solid food*	n=3 (10)	n=6 (7)	n=4 (7)	

Table 4.15: Infant characteristics at a) birth and b) visit 4 based on feeding mode. Body composition at 5 months revealed that BF infants had lower FFM, after adjusting for infant size.

* Data indicate the number of participants who introduced solid food before the follow up assessment, data in () indicate the mean duration of solid food intake in days, before the follow up assessment

4.3 Discussion

We have presented a comprehensive analysis of the maternal and infant characteristics predicting body composition showing the following key findings:

1. Increase in fetal weight during the late stages of gestation is driven by FFM, rather than FM, accumulation;
2. Maternal BMI and GWG independently predict offspring adiposity during the first 5 months of life;
3. Sex differences in body composition are present at birth;
4. BF infants tend to have lower weight gain but higher %FM and lower FFM at 5 months post partum and
5. Infants of SA origin have similar body composition with infants of European descent.

4.3.1 Maternal BMI, gestational weight gain and gestational age

The increasing maternal obesity rates parallel the childhood obesity epidemic raising the question of developmental programming (27). Data from multiethnic cohorts reveal that maternal obesity is related to increased childhood and adulthood adiposity (4, 5). Accumulating evidence suggest that this adverse phenotype is present at birth, with offspring of mothers with overweight and obesity having higher FM compared to those born to women of a normal weight (28, 29). At the same time, well designed randomized control trials have shown that GWG is inversely related to offspring adiposity at 6 months of age. Observational studies have shown that these adverse outcomes are maintained in childhood and adulthood (8, 30, 31).

During pregnancy, adipose tissue is used as storage which continuously provides energy to the fetus, matching the requirements of intrauterine growth. Maternal fat accumulation parallels GWG (32), with adipose tissue being predominantly centrally deposited (Figure 4.6). Central adiposity is linked to reduced insulin sensitivity and hyperlipidaemia, even in pregnancy (33). Insulin resistance leads to hyperglycemia and hypertriglyceridaemia due to unopposed lipolysis. In cases of preexisting maternal obesity, the above effect is exaggerated due to increased peripheral insulin resistance (34). As a result, maternal obesity and high GWG lead to excessive transplacental transport of nutrients (glucose, free fatty acids and amino acids) to the fetus, stimulating fetal insulin secretion. The chronic fetal hyperinsulinaemia leads to macrosomia and FM accumulation.

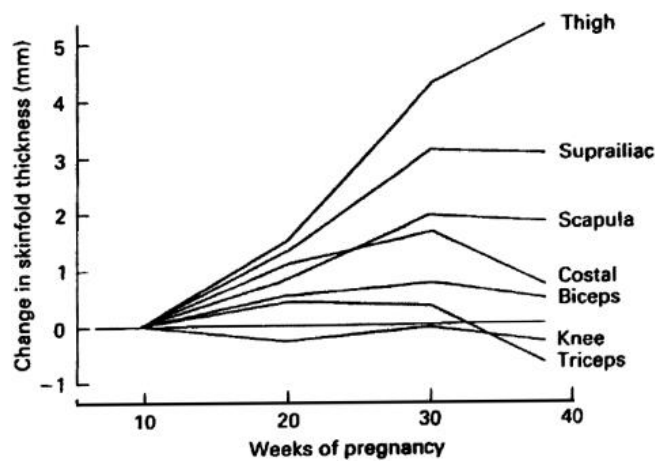


Figure 4.6: Changes in skinfold thickness during pregnancy. Graph demonstrates the preferential central adipose tissue deposition; Adopted from Taggart et al (35)

Using an objective body composition technique (PEAPOD) to describe offspring adiposity, we managed to demonstrate that maternal BMI and GWG are related to higher adiposity at birth and up to 5 months of age, with BMI having a greater effect. Similarly to the effect of GDM on body composition, we believe that the small effect size noted may be due to a delayed effect, which will become more prominent after 5 years of age (36). Furthermore, evidence from the Project Viva has shown that modest differences in body composition during the early postnatal period may be amplified as child gets older (37). Our data also show that GWG is independently related to FFM. To date, there is paucity of data regarding the metabolic significance of FFM at the early stages of life as most studies focus on the quantification of FM. We suggest that FFM is an important component of metabolic imprinting and more studies are required to clarify its association with maternal characteristics. Specifically, we believe that GWG below the IOM recommendations could lead to compromised FFM development contributing to adverse cardio-metabolic outcomes in later life. We were unable to demonstrate any association between maternal BMI and FFM at birth. We speculate that the weak association between BMI and FFM during the first 5 months post partum is driven by the higher weight of the offspring of mothers with obesity.

As expected, we have shown that increasing gestational age is related to higher weight, FM and FFM. Consistent with the Newborn Body Composition Study (Intergrowth 21st) (38) data we have shown that the rate of FFM accumulation is higher than that of FM during the late stages of pregnancy. After assessing 1000 infants, Intergrowth study team showed a mean increase of FM by 35gr/week and FFM by 162gr/week after 34 weeks of gestation, with great variability between subjects. Although the effect size on both FM (14gr/week) and FFM (63gr/week) is different between the two studies, the ratio of

FFM/FM accretion with increasing gestational age is similar. Furthermore, our study involved participants with high metabolic risk whereas the Intergrowth sub study included low to medium risk pregnancies (mean BMI in our study: $29.8 \pm 7 \text{ kg/m}^2$ vs. Intergrowth BMI: $24.9 \pm 4.9 \text{ kg/m}^2$). The exact effects of maternal obesogenic intrauterine environment remain unknown, therefore comparison of the two groups by means of absolute FM and FFM values may not be reliable.

4.3.2 Body composition and gender

We performed a subgroup analysis to assess gender differences in body composition during the early stages of life. We used PEAPOD as a direct measure of body composition to describe differences in FM and FFM between sexes. We reviewed data from fetal anomaly scans at 20 weeks of gestation to identify any early impact of gender on fetal growth and we used skinfold thickness to assess any difference in FM distribution in our study groups. Our results show that gender dimorphism in body composition is present at birth but not during early pregnancy. We found that females have a more adipose phenotype with higher %FM, higher FMI and lower FFM. We were unable to demonstrate any difference in adipose tissue distribution during the first 5 months of life. Our results are consistent with findings from two large studies which also used PEAPOD technique to assess neonatal body composition. The Intergrowth 21st study team (38) assessed body composition in 1019 infants born in Oxford, UK, after 34 weeks of gestation. Norris et al (39) combined data from 4 different multi ethnic (US, Ireland, Italy) cohorts (n= 1457) in order to create body composition charts from 30 weeks of gestation up to 6 months post partum. Both studies showed that females have consistently higher %FM and lower FFM when compared to males.

Gender differences in body composition are well described in adult populations. Females are known to have higher %FM and lower FFM for a given BMI. Despite the higher total adiposity females have lower cardiometabolic risk which is related to the adipose tissue distribution (40). Males have central adiposity whereas females are characterized by the “pear shaped” phenotype, with subcutaneous fat accumulation around the pelvic region (13). Body composition differences arise from sex chromosomes and sex hormones (14). In order to distinguish the contribution of chromosomes and hormones to adipose tissue formation animal models have been used. In mice models, in an attempt to eliminate the gonadal effect, the testis determining gene (Sry) was deleted from the Y chromosome and a transgene was inserted to an autosome. As a result, four core genotype models were created: XX mice with either male or female gonads and XY mice with either male

or female gonads (Figure 4.7). Presence of the XX genotype was related to higher total body fat and greater appetite. The same genotype when exposed to high fat diet demonstrated larger subcutaneous inguinal adipose tissue deposition whereas XY mice accumulated adipose tissue around the gonadal fat pods (visceral fat) (14).

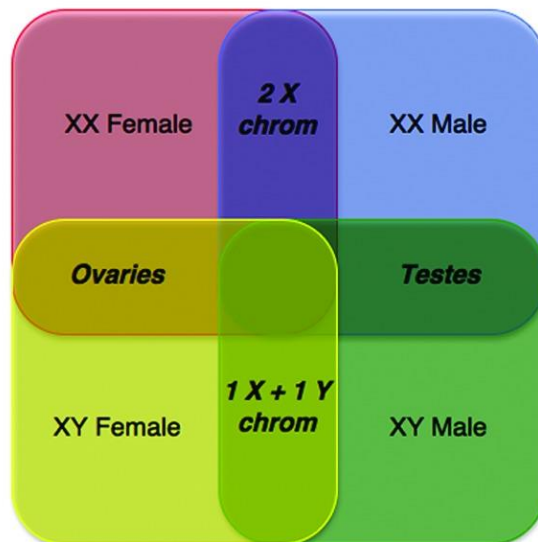


Figure 4.7: The four core genotypes mouse model. Differences between gonadal females and gonadal males are attributed to sex hormone effects, while differences between XX and XY mice are attributed to the sex chromosome; Adopted by Link et al (14)

The role of sex hormones has been widely described (Figure 4.8). Various expression of oestrogen receptors within different depots of WAT is responsible for adipose tissue distribution. Oestrogen receptors are predominantly expressed in subcutaneous rather than visceral fat. Furthermore, gluteal subcutaneous fat has more receptors than abdominal subcutaneous, which explains the preferential deposition of adipose tissue around the pelvic region in females (41). To further support this theory, postmenopausal women have increased central adiposity when compared to premenopausal. This relation is attenuated in postmenopausal women receiving hormone replacement therapy (42). Finally, in cases of PCOS which are characterized by an imbalance between oestrogens and testosterone, women present with higher visceral fat (43).

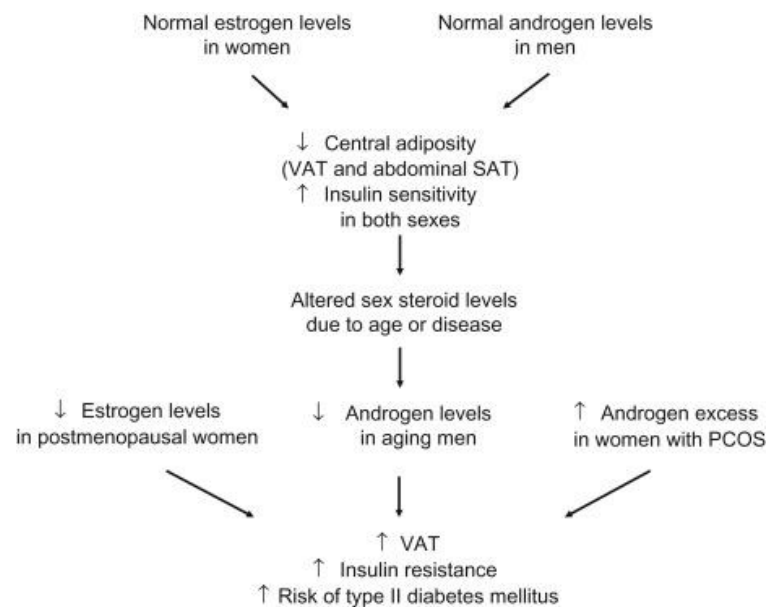


Figure 4.8: Sex hormones, body composition and cardiovascular risk; Adopted from Ethun et al (44)

In our cohort, we demonstrated higher adiposity in female infants. Consistent with animal studies described before, we believe that these differences are driven by the sex chromosomes. We were unable to find any difference in adipose tissue distribution. As previously explained, adipose tissue depot formation is strongly linked to sex hormones. We believe that the sexual dimorphic pattern of reproductive hormone levels described during early infancy (45) is not strong enough to create different patterns in adipose tissue deposition. To further support our hypothesis, Kanehisa et al (46) has previously shown that mild body composition differences between sexes in childhood are amplified during puberty, a period of “sex hormone prosperity”.

4.3.3 Body composition and feeding mode

We utilized data from our observational study to compare body composition based on different feeding modes. Consistent with national data (19), 36.5% of our participants were found to predominantly BF at 4 weeks and only 22.6 at 5 months. Similarly to Oddy et al (47) we observed that maternal BMI was associated with reduced duration of BF. In our cohort, BF was related to lower weight gain but higher %FM and lower FFMI at 5 months.

The long term benefits of BF on non-communicable diseases are well established. Data from seven systematic reviews, including 81 studies, has shown that BF reduces the risk of childhood obesity (48). Based on this evidence, BF has received the unanimous support from many international organizations (CDC, WHO, NICE). The exact

mechanism underpinning the protective effect of BF is yet to be identified. The number of studies on non-nutritional components of milk, such as leptin, adiponectin, insulin and ghrelin has been progressively increasing in the last decade, but the causal link to metabolic outcomes remains unknown (48).

At the same time, data on the effect of breast milk on infant body composition remains controversial. In our study we showed that BF infants have lower weight gain at 5 months. The reduced concentration of energy, protein and micronutrients in breast milk compared to formula can explain the above findings (49, 50). Consistent with our results, Butte et al (22) and Anderson et al (23), using DXA and PEAPOD respectively, have shown that BF infants have higher %FM and lower FFM during the first months of life. In contrast, results from UK (51) and Italian (52) cohorts suggest that BF is related to consistently lower weight gain, lower BMI and FM up to 5 months of age. We believe that the higher energy density of formula milk and specifically the higher protein/energy ratio may be responsible for the lower FFM observed in BF infants (53). We hypothesize that the increased adiposity observed in BF infants may be related to bioactive components of breast milk, such as leptin, adiponectin and insulin. For example, maternal BMI is related to breast milk insulin concentration (54). Given the preferential effect of insulin on FM, we speculate that levels above a normal “cut off” could potentially lead to increased FM accumulation. Overall, current literature is sparse and the exact effects of breast milk are difficult to be established. There are both known and unknown factors influencing maternal breast milk composition, and these factors may, in turn, affect infant outcomes independent of their effect on breast milk composition (48). More studies are required to explore these hypotheses to ascertain the link between breastfeeding and early childhood body composition. This may help to understand its beneficial effects on metabolic disorders in latter life.

Interestingly, one of the most recent studies suggested that breast milk is not related to the quantity of adipose tissue but the type (55). In specific, researchers have shown that lipids [alkylglycerol-type (AKG-type) ether lipids] present only in breast milk delay the conversion of beige to white adipose tissue which usually takes place during late infancy. Knowing that beige adipose tissue is inversely correlated to childhood obesity, authors have suggested that the prolonged presence of the metabolically active beige adipocytes could be the causal link between BF and reduced future obesity. To further investigate this hypothesis, studies using glucose uptake scans will be required as other body composition techniques (PEAPOD, DXA, MRI) are unable to differentiate between various types of adipose tissue.

There are several limitations in the studies described above, including our study. Observational studies are not able to infer causation, therefore well designed RCTs would be required to assess the true effect of BF. These would be unethical when the weight of evidence for BF as being beneficial for both mother and child is considerable, and because, carrying out RCTs during the perinatal period is very sensitive. The definition of BF varies between different studies, therefore comparison of the results may not be reliable. In our cohort we categorized participants as predominantly breastfeeding (BF), predominantly formula feeding (FF) and mixed feeding based on the total duration of feeding mode over a period of 5 months (e.g. mixed: similar proportions of breast and formula milk). We were unable to capture data regarding the number of participants who fed their infants pumped breast milk. This could have potentially biased the results as pumped breast milk is also coming under the category of BF without however the non-nutritional benefits of BF, such as skin to skin contact, slower suckling rate and improved satiety. Furthermore, data on feeding mode is gathered based on maternal report. Cultural, religious and social stigma could potentially lead mothers to false reporting of feeding patterns. Finally, the inability to control for confounders is a common critique of available literature. In our trial, due to the size/weight limitations of PEAPOD, participants were followed only up to 5 months post partum. Only a very small percentage (8.6%) of our cohort reported introduction of very small amounts of solid food by that point (irrespective of ethnicity), which ensures that our data is free from the confounding effect of caloric intake through solid food.

4.3.4 Body composition and ethnicity

Our study presents unique data on body composition based on ethnic origin. We showed that SA and White European infants have similar body composition and adipose tissue distribution up to 5 months of age. This is contrary to existing literature which show that SA infants have higher adiposity than White Europeans (16).

SA are at higher risk of type 2 diabetes and cardiovascular disease than populations descended from White Europeans (56). They are known to have higher FM and lower FFM for a given BMI when compared to other populations (57). Furthermore, they have a tendency to store adipose tissue centrally (visceral fat) rather than peripherally (subcutaneous fat), which increases their cardiovascular risk further (15). This adverse body composition has been shown to be present in childhood and even at birth (58, 59). Interestingly, this increased metabolic risk has been seen in SA migrants around the world (60). In UK, diabetes prevalence is five times higher for SA population and the

onset is ten years earlier than native population (56). UKPDS suggested that increased insulin resistance is the main cause of the observed cardiometabolic risk (61). It was previously suggested that central adiposity is the link between ethnic origin and increased risk of insulin resistance. The above hypothesis is not enough to explain the pathophysiology observed as SA continue to exhibit higher insulin resistance even if they are matched for central adipose tissue amount (62). The above results led to the assumption that SA are genetically predisposed to have adverse metabolism as compared with people descended from white European populations. A recent systematic review on ethnic genetic heterogeneity using global data from DIAGRAM (Diabetes Genetics Replication And Meta-analysis) showed that presence of nucleotide polymorphisms predisposes SA to type 2 diabetes (Figure 4.9) but evidence is not strong enough to indicate higher genetic risk compared to White Europeans (63).

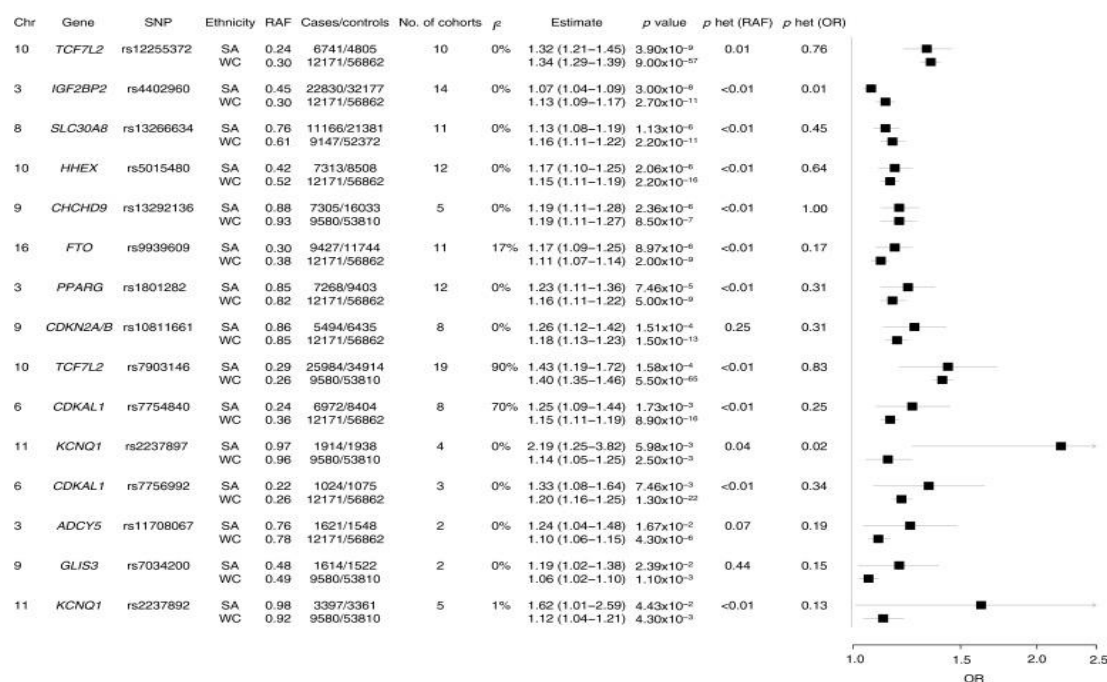


Figure 4.9: Nucleotide polymorphisms associated with type 2 diabetes mellitus in SA and risk compared to White Europeans. KCNQ1 seems to have the strongest correlation; Adopted by Sohani et al (63)

When studying the association between migration and non communicable diseases there are many factors that need to be taken into account (Figure 4.10). Except genes, exposures of subjects in pre-migration need to be carefully reviewed. In specific, the role of intrauterine exposure to malnutrition and its adverse effect on future metabolic diseases need to be considered, as the metabolic imprinting can be profound for many generations (64, 65). Furthermore, eating habits, social and religious beliefs may be

retained by migrants for many years before becoming acculturated to local customs and way of life (66). Therefore, although someone may consider that both native and migrant populations are exposed to the same environment, this may not be entirely true. Finally, data in the past has shown that migrants had limited access to education and healthcare services, suggesting that lower screening rates and preventative measures could be potential reasons for the higher mortality amongst these populations (67).

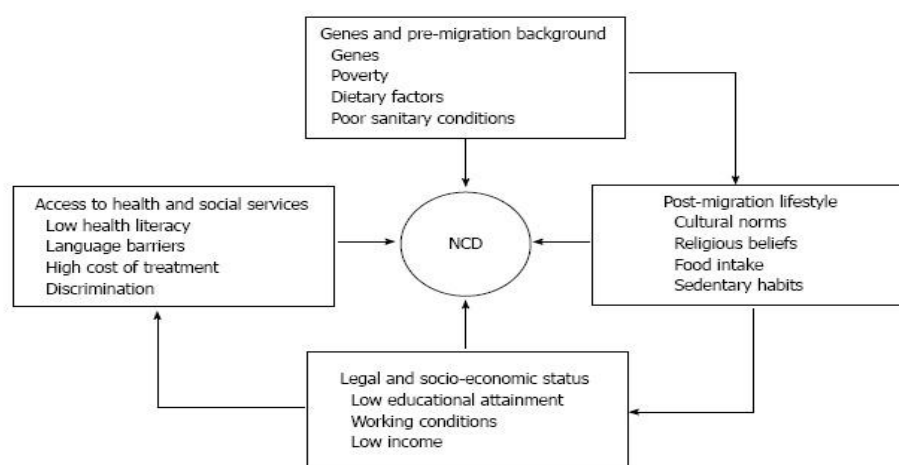


Figure 4.10: Factors associated with non communicable diseases in migrants. Genes and pre-migration status as well as post-migration lifestyle dictate the risk for non-communicable diseases (NCD); Adopted from Montesi et al (68)

Yajnik et al (16, 59) has extensively investigated body composition of SA infants living in India. After comparing newborns in Pune, India with White Europeans born in the UK concluded that SA infants are more centrally adipose resulting in increased insulin resistance from the early stages of life. These findings were further confirmed by Modi et al (69) who used MRI to show increased visceral and reduced subcutaneous adiposity in infants born in Asia compared with White European newborns in UK. These findings are not comparable with the results of our cohort. The above-mentioned studies, reviewed a high risk population prone to maternal malnutrition giving birth in Pune, India. Findings of these studies could be explained by the “thrifty phenotype” model which is closely related to maternal nutritional status (70). Our cohort consists of SA population with completely different maternal characteristics, both anthropometric (higher BMI and height) and socioeconomic (higher educational and socioeconomic status). Furthermore, antenatal services were provided in the NHS which allows closer monitoring during the antenatal period.

The first large cohort to compare body composition differences between migrant and native population was published in 2009 (17). The study team assessed body composition in infants born in Pune, infants of SA origin born in Surinam (South America) and infants born to the population native to Surinam. Findings suggested that the “thin-fat” phenotype was still present in 4th-5th generation migrants but body composition differences with native population were attenuated when compared with infants born in Pune. In 2012, The London Mother and Baby study (71) assessed body composition differences between UK born infants of SA origin and White European infants using PEAPOD, showing that SA have lower FFM and higher central adiposity at 8 weeks post partum. The team suggested that the lower FFM is the potential link to the future adverse cardiometabolic risk. Our study cohort has many similarities with the Surinam study. Maternal BMI (our study: 23.7, Surinam: 23.4), maternal diet patterns (majority non vegetarians), neonatal birthweight (our study: 3.090 kgr, Surinam: 3.159) and length (our study: 49 cm, Surinam: 48.7 cm) were similar between the SA populations. The authors of Surinam study describe the community of SA migrants as “closely knit” and genetically homogenous. Furthermore, the authors do not provide any data on the socioeconomic status of the participants which is relevant to the access to the local health system. We speculate that the difference in findings from our cohort is due to lifestyle and eating habits which may create an obesogenic intrauterine environment. We also question whether study participants had full access to healthcare services, including regular screenings, diet advice and educational sessions in the Surinam study. Regarding the London cohort we are able to identify many differences with our study which could explain the results. Mothers of SA origin in the London cohort were significantly shorter (our study: 1.65 m, London: 1.58) compared to our study. When compared to White Europeans, SA mothers were found to be more adipose in the London cohort (SA: SS=21, TR= 26.5 mm; Europeans: SS= 17, TR= 22.2 mm) whereas there was no between groups difference in our study. Of great importance half of the mothers were vegetarians as opposed to only 5% in our cohort. Finally, there was a statistical significant difference in GA between the two groups (SA: GA=39.4 weeks; Europeans: 40 weeks, $p<0.05$), which was not observed in our study. We have previously showed in our study that maternal adiposity is related to offspring FM and also that lower gestational age is related to lower FFM at birth. Using data from the Minnesota experiment we proved that lower FFM can lead to rapid weight gain driven by predominantly FM accumulation (Chapter 3). Vegetarian diet is linked to low B12 levels and reduced protein intake (72). Given the link of low maternal B12 with offspring adiposity and insulin resistance we suggest that maternal diet could have directly affected neonatal body composition. Finally, low protein intake could have potentially contributed to lower FFM seen in SA infants (73).

In one of the largest cohorts so far, cord leptin levels and skinfold thickness were compared between 4,649 Pakistani and 4,055 White British neonates born in Bradford, UK (74). Authors concluded that based on adjusted cord blood leptin levels offspring of Pakistani origin were more adipose at birth. Large sample size and definition of offspring's ethnicity taking into account both maternal and paternal place of birth were the major strengths of the study. A closer look at the results though, puts the credibility of the conclusion under question. After adjusting for birthweight, authors were unable to show any between group differences in skinfold thickness. The difference in cord leptin levels was demonstrated after adjusting for birthweight. The higher leptin levels found in the Pakistani group doesn't necessarily imply higher FM at birth. The raised leptin levels may be due to increased leptin expression by adipocytes as a result of adverse epigenetic programming. In favor of this hypothesis, authors present data revealing that differences in cord leptin levels attenuate (or even become non significant) after adjusting for maternal characteristics which could potentially affect intrauterine programming (BMI, height, socioeconomic status). The Born in Bradford cohort has significant demographic differences to our study group. Mothers were only from Pakistani origin, had low socioeconomic status, had higher BMI and were shorter when compared to the SA population of our study.

Findings of our study are in agreement with the Intergrowth 21st (75) and WHO multicentre Growth Reference study (76). After analyzing fetal and infant data across 8 different populations it was suggested that growth is affected more by health, socioeconomic and environmental factors rather than ethnic and genetic characteristics which account only for <3% of total variability. Based on this evidence the above committees suggested universal growth standards in low risk populations, as defined by demographic, clinical, social and educational criteria. Our SA cohort shares many similar characteristics with the "low risk" Indian population studied in the Intergrowth trial (Table 4.16). Maternal BMI was marginally higher in our site and mothers were taller. Birthweight was slightly higher in our cohort, likely due to higher maternal BMI. Birth length and HC at birth were identical to Intergrowth data suggesting that fetal skeletal/FFM growth was not compromised.

	SA in our cohort	Intergrowth 21 st
Maternal BMI (kgr/m²)	23.7	22.9
Maternal Height (m)	1.65	1.57
Birthweight (kgr)	3.065	2.900
Birth length (cm)	49	48.6
HC (cm)	33.9	33.1

Table 4.16: Comparison of South Asian (SA) population participating in our cohort and Intergrowth 21st. In our study cohort mothers of SA origin were taller with higher BMI resulting in higher birthweight

Our cohort involves participants of SA origin with good socioeconomic and educational status. 40% of the participants (mothers) were born in the UK (first generation migrants) and the remaining moved into the country at a very young age. The high educational status of our cohort may have raised the awareness of maternal imprinting to offspring, thus leading to healthier lifestyle options during the pre and antenatal periods. Of great importance, NHS has evolved during the last few years and a health policy regarding immigrants has been created (77). Ethnic minority groups share equal rights and time during consultations and the presence of translators, when required, ensures that all information provided is retained by the patients. Furthermore, leaflets translated in various languages have helped to raise awareness regarding the obesity epidemic and ways to prevent it. The high stature of our participants confirms the “healthy migrant effect”. According to this SA with greater financial means for maternal and child nutrition are more likely to migrate. The height of our participants reveals that they were free from the “maternal constraint effect” (78) as fetuses, in other words mothers of our cohort experienced an optimal intrauterine environment as fetuses. The similar total adiposity and adipose tissue distribution compared to native population is another clue of the non-obesogenic intrauterine environment.

In conclusion, we strongly believe that despite genotype being determined at conception, the phenotype is modulated and determined by environmental factors known as epigenetic mechanisms. Imprinted genes can be modified by environmental factors acting during the “plastic” period of preconception to early post natal life. Maternal behaviors can affect structural and functional development of tissues and determine the transgenerational transmission of non communicable diseases. As epigenetic effects may not be evident until later in life, it would be of great interest to examine the evolution of our cohort into childhood and adulthood.

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Chapter 5

Maternal, cord blood biomarkers and infant adiposity

5.1 Introduction

In our previous chapters we have demonstrated that adverse maternal characteristics, such as obesity, increased GWG and GDM are associated with increased infant adiposity up to 5 months post partum. Our results show that the link between adverse intrauterine environment and risk of future metabolic diseases is traced back to the early postnatal life, supporting the theory of intrauterine programming. Our findings suggest that intervention strategies to prevent the development of obesity and metabolic syndrome should be implemented during antenatal or even prenatal life, a period of developmental plasticity. Finding the link between maternal and fetal biomarkers with adverse infant anthropometry would allow a better understanding of the pathophysiology of intrauterine programming and would enable the identification of high risk individuals requiring targeted approach.

5.1.1 Fetal anabolic fuels

In 1952, Pedersen postulated that maternal hyperglycaemia is transferred to the fetal circulation via the placenta, which in turn stimulates fetal hyperinsulinaemia. Given the anabolic action of insulin and the preferential effect on FM, excessive exposure of the fetus to insulin leads to deposition of large amounts of body fat and neonatal macrosomia (2). The HAPO study, in a multinational cohort, confirmed the “Pedersen hypothesis” by showing that maternal glycaemia during OGTT is related to infant adiposity and macrosomia, a relationship mediated by increased fetal insulin (3). As maternal BMI is related to higher fasting and postprandial glucose levels (4) the HAPO research group tried to investigate if the observed relationship was driven by the effect of maternal BMI rather than hyperglycaemia per se. Data revealed that both maternal obesity and hyperglycaemia are independent risk factors of neonatal adverse outcomes with their combination having a stronger impact (5, 6). The independent relationship between maternal BMI and neonatal size highlighted the importance of nutrients other than glucose. Lipids, free fatty acids and amino acids which are also related to insulin resistance and are found to be raised in mothers with high BMI (7) may contribute to neonatal fat accretion (8). Data from animal studies and cell lines reveal that maternal adiponectin inhibits placental proliferation and attenuates the trophoblast transporter expression (9). The association between maternal obesity and hypoadiponectinaemia may lead to placental hypertrophy allowing more nutrients to fetal circulation and determine the fetal size (10).

5.1.2 The role of leptin and adiponectin

The role of leptin and adiponectin in human metabolism and during pregnancy has been extensively described in chapter 2. Leptin promotes energy expenditure by inhibiting lipogenesis and glycogenesis and by enhancing free fatty acid oxidization. Levels are positively correlated with adiposity and maternal BMI (11). On the other hand, adiponectin improves insulin sensitivity and low levels are observed in non-pregnant adults with type 2 diabetes mellitus and obesity (12). Data suggests that high molecular weight adiponectin (HMWA), rather than total, is implicated in the risk of metabolic syndrome, insulin resistance and cardiovascular disease in adults and children (13). Both leptin and adiponectin are present in fetal circulation from the early stages of pregnancy. The presence of mRNA and their receptors in many fetal tissues implies a role during intrauterine development (14, 15). Results of our systematic review, although not conclusive, revealed that both adipocytokines are positively correlated to adiposity at birth, with cord leptin levels negatively predicting weight gain and fat mass accretion up to 3 years of age.

5.1.3 Hypothesis

Mid gestation maternal biomarkers reflect maternal metabolic profile and intrauterine environment. While HAPO study concluded that maternal glucose levels and BMI at 28 weeks of gestation were independently associated with infant adiposity and is likely to be mediated by increased fetal hyperinsulinaemia, the role of maternal insulin, leptin and adiponectin were not investigated. In addition the effects of these on FFM is not known. We hypothesise that the following two relationship exist between: 1) maternal BMI may mediate infant adiposity through hyperleptinemia, low adiponectin and raised insulin levels; and 2) cord blood leptin and adiponectin are reliable markers of neonatal adiposity at birth and early childhood (up to 5 months of age). If proven, these biomarkers may enable to identify children who are at risk of adverse metabolic intrauterine programming and develop strategies to minimize the risk.

5.1.4 Research Questions

- Is there any association between mid gestation maternal biomarkers (leptin, adiponectin, insulin, glucose) and infant body composition?
- Is there any association between cord leptin and adiponectin with body composition, assessed by air displacement plethysmography (PEAPOD), at birth and up to 5 months of age?

5.1.5 Outcomes

- Study if different patterns of neonatal body composition are related to mid-pregnancy maternal metabolic markers (leptin, adiponectin, insulin, glucose) and early life biomarkers (cord blood leptin and adiponectin)

5.1.6 Criteria

- The current analysis includes participants from the main longitudinal, observational study described in chapter 3. For this reason inclusion and exclusion criteria remain the same as already described before

5.1.7 Methods - Statistical analysis

Paired maternal and infant data from the main observational, longitudinal cohort study was utilized. Maternal samples obtained during the 75 gr OGTT at 26-28 weeks of gestation were retrieved. Baseline samples during the OGTT (OGTT₀) were analyzed for maternal leptin, adiponectin, glucose, insulin and c-peptide. Samples obtained 2 hours after the 75 gr glucose load (OGTT₁₂₀) were analyzed for glucose, insulin and c-peptide. We utilized data obtained from the OGTT analysis to calculate beta cell insulin secretory capacity and insulin resistance using HOMA₂ and HOMA-IR/ Matsuda index respectively. Cord blood samples were tested for leptin, adiponectin and c-peptide levels. Infant body composition was assessed at birth, 4 weeks and 5 months post partum using PEAPOD.

5.2 Leptin- Adiponectin assays

Leptin and adiponectin levels in both maternal and cord samples were measured using Enzyme-Linked Immunosorbent Assay (ELISA). This technique allows the detection and quantification of an antigen (protein) by using antibodies (Abs) directed against the antigen (Figure 5.1). All analyses were performed at the Clinical Sciences Research Laboratories (CSRL) at University Hospital of Coventry.

5.2.1 Leptin Elisa

All samples were removed from the freezer (-80°C) and allowed to rest at room temperature for at least 30 minutes. 10 µL of sample were retrieved and analyzed using ‘sandwich’ ELISA (Quantikine ELISA, R&D systems, 3.5 hours assay). Leptin specific Abs had been pre-coated in each well. After the addition of the sample, leptin molecules were bound to the immobilized Abs. The plate was then washed to remove any unbound Abs and enzyme linked Abs specific for leptin were added to each well. The addition of

the new Ab allowed the “sandwich reaction” due to the ability to bind to different leptin epitopes. The plate was then washed to remove any unbound enzyme linked Abs. Finally, a substrate (chromogen) was added to each well which was linked to the enzyme, resulting in a colour development (positive reaction) in proportion to the amount of bond leptin. The optic density of each plate was then measured using microplate reader at 450nm (spectrophotometer).

5.2.2 Adiponectin Elisa

Adiponectin was measured using “sandwich” ELISA (HMW and Total adiponectin ELISA, ALPCO, 4.5 hours assay). The same process described before was followed for adiponectin quantification. In addition, in order to quantify HMWA, a treatment enzyme (protease) to degrade total to HMWA (“digest low and medium weight adiponectin) was added to each sample.

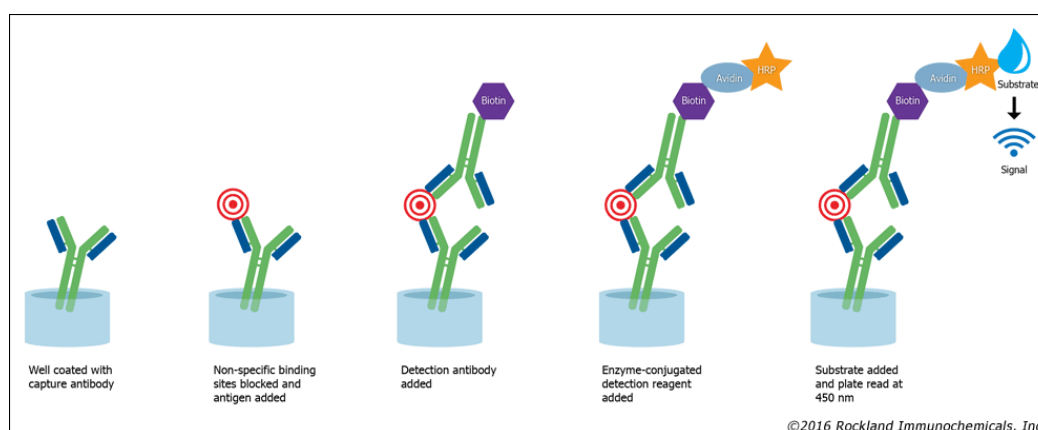


Figure 5.1: “Sandwich” ELISA technique; Adopted from Rockland Immunochemicals Inc., 2016

5.2.3 Calculating the results

In order to extract the results a “standard curve” from the standards was created for each plate. The mean absorbance of the standards was plotted on the y axis and the concentration on the x axis. A best fit curve was fitted. For the leptin ELISA the curve was linear whereas for the adiponectin a 4 Parameter Logistic (sigmoid) curve was used. Concentrations extracted from the standard curves were multiplied by the dilution factor (100 for leptin, 5000 for adiponectin) in order to get the concentration present in each sample. Intra and inter assay co-efficient of variation was <5% for leptin and <10% for the adiponectin assay (Figure 5.2).

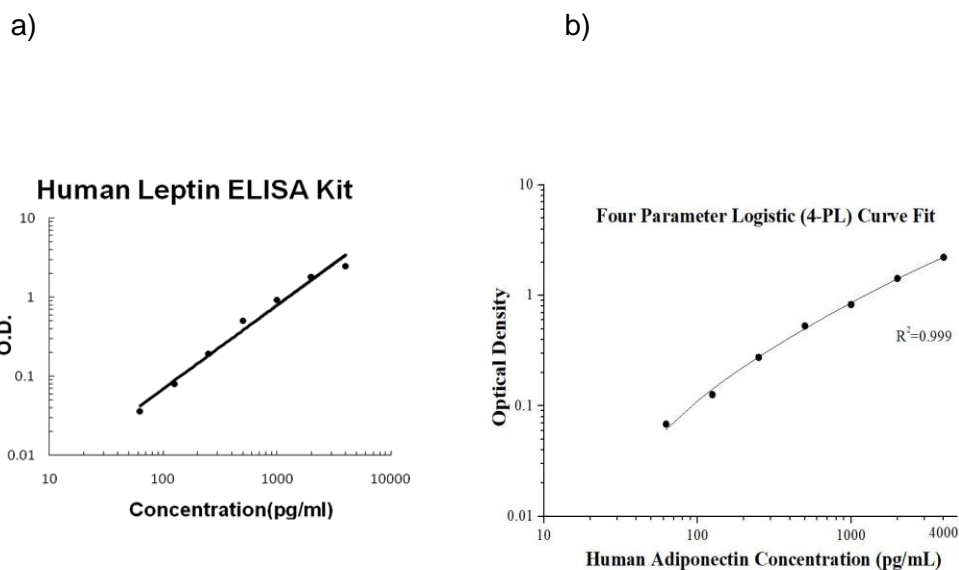


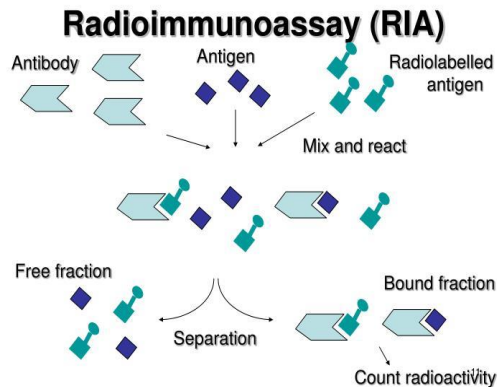
Figure 5.2: Standard curves fitted for a) leptin and b) adiponectin

5.3 Insulin and c-peptide assays

Maternal samples were analyzed for both insulin and c-peptide whereas cord samples only for c-peptide. We chose to use cord blood c-peptide as a marker of fetal beta cell function rather than insulin, as c-peptide is not affected (degraded) by haemolysis which is present in 15% of cord samples (16). C-peptide is an amino acid connecting the insulin A and B chains in the proinsulin molecule. Proteolysis of proinsulin results in equal amounts of insulin and c-peptide formation. Insulin, which is the active product, is metabolized in the liver having a short half life of 3 minutes. On the other hand, c-peptide, the inactive para-product, is not extracted in the liver resulting in longer half life (35 minutes) and ten times higher plasma concentrations (17).

Measurements of both insulin and c-peptide were performed using “sandwich” (non competitive) Radioimmunoassay (RIA) at George Eliot Hospital biochemistry department. Catherine Wood, principal clinical biochemist, was supervising the process. RIA is the technique of using radiolabelled Ag/Ab complexes to determine the concentration of an Ag (Figure 5.3). ROCHE assays were used for both insulin (Elecsys Insulin, cobas) and c-peptide (Elecsys c-peptide, cobas). Inter and intra assay co-efficient of variation was <5% for both assays.

a)



b)

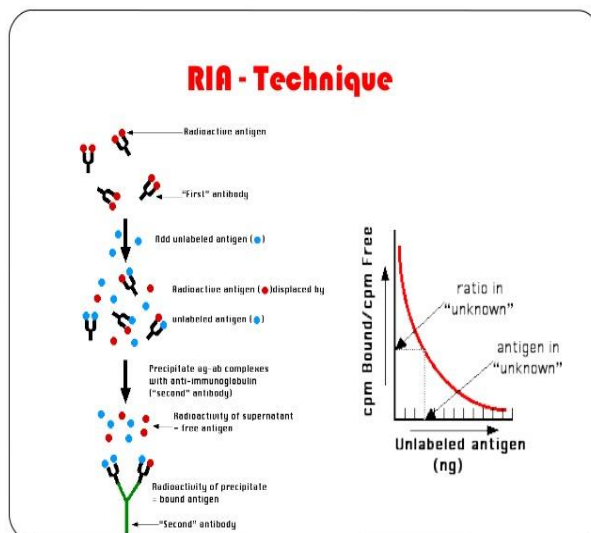


Figure 5.3: Principles of a) non competitive and b) competitive RIA

5.3.1 Insulin and c-peptide RIA

Standard curves were created using known quantity of radioactive Ag mixed with known amount of Ag specific Ab. The first step was to mix 20 μL of sample with Ag specific Ab and labeled Ag specific Ab in order to form a "sandwich reaction". In order to separate the Ab bound Ag from the free particles, streptavidin microparticles were added to the solution allowing the Ab-Ag-labelled Ab complex to bind to the solid phase. The microparticles were then magnetically captured on the surface of an electrode and the radioactivity of the complex was measured using gamma sensors. Using the standard curves created we were able to calculate the concentration of the Ag (insulin or c-peptide) in our sample.

5.4 Indexes of beta cell function and insulin sensitivity

Insulin sensitivity or resistance underlies the development of type 2 diabetes or any degree of glucose intolerance. Elements of insulin sensitivity are the absorption of glucose by the peripheral tissues and the insulin mediated suppression of hepatic glucose production. Hyperinsulinaemic – euglycaemic clamp (HEC) is considered the “gold standard” method in assessing peripheral insulin sensitivity but the method is costly, invasive and not appropriate for large scale studies. For this reason many indexes based on either fasting or OGTT derived glucose/insulin levels have been created (Table 5.1). Fasting indexes reflect hepatic insulin sensitivity whereas OGTT derived reflect both hepatic and peripheral sensitivity. During the OGTT, hepatic glucose production is more prominent within the first hour and glucose uptake by peripheral tissues during the second hour (18). In our study, maternal glucose and insulin/c-peptide levels were available at OGTT₀ and OGTT₁₂₀. We opted to use both a fasting (HOMA-IR) and an OGTT derived index (Matsuda index) to assess insulin sensitivity in our cohort. Both indexes used have a high correlation with reference HEC (HOMA-IR: $r=-0.6$, Matsuda: $r=0.67$) (19, 20).

OGTT based indexes	Equation
Stumvoll MCR (21)	$18.8 - 0.271 \times \text{BMI} - 0.0052 \times I_{120} - 0.27 \times G_{90}$
Gutt (22)	$[75,000 + (G_0 - G_{120}) \times 0.19 \times \text{BW}] / (120 \times \log [(I_0 + I_{120})/2] \times [(G_0 + G_{120})/2])$
Stumvoll ISI (21)	$0.226 - 0.0032 \times \text{BMI} - 0.0000645 \times I_{120} - 0.00375 \times G_{90}$
OGIS (23)	http://webmet.pd.cnr.it/ogis/
Matsuda (24)	$10,000 / \sqrt{G_0 \times I_0 \times G_{\text{mean}} \times I_{\text{mean}}}$
Fasting indexes	
HOMA-IR (25)	$(G_0 \times I_0) / 22.5$
QUICKI (26)	$1 / (\log G_0 + \log I_0)$
Revised QUICKI (27)	$1 / (\log G_0 + \log I_0 + \log \text{NEFA})$
HOMA-%S (25)	www.dtu.ox.ac.uk/homacalculator/index.php
FIRI (28)	$(G_0 \times I_0) / 25$

Table 5.1: Surrogate measures of insulin sensitivity. Fasting indexes reflect hepatic insulin sensitivity whereas OGTT derived reflect both hepatic and peripheral sensitivity; Adopted from Otten et al (19); I_0 and I_{120} : insulin levels at baseline and 120 minutes after 75gr glucose administration; G_0 , G_{90} and G_{120} : glucose levels at baseline, 90 and 120 minutes after 75gr glucose administration; G_{mean} and I_{mean} : mean glucose and insulin levels during OGTT

5.4.1 Homeostasis Model Assessment (HOMA)

HOMA estimates pancreatic beta cell function (%B) and insulin sensitivity (%S) as percentage of a normal population. Model is based on the Turner and Holman assumption (1) that fasting glucose and insulin levels are determined by a hepatic-pancreatic interaction (Figure 5.4). Healthy population and even patients with type 2 diabetes mellitus (mild forms) have equal fasting insulin levels but fasting glucose levels vary at the individual level. Fasting hyperinsulinaemia is in proportion to increased insulin resistance whereas fasting hyperglycaemia is the “sensor” to stimulate deficient pancreatic cells to secrete normal basal insulin. In other words, fasting glucose reflects the pancreatic secretory capacity and fasting insulin reflects the insulin resistance (Figure 5.5).

In 1985, Matthews et al (25) expanded the model producing a linear equation of %B and introduced the term of insulin resistance (HOMA-IR) as the reciprocal of %S ($100/\%S$). Ten years later, Levy et al (29) implemented in the formula values obtained by more advanced insulin/c-peptide assays and accounted for variations in hepatic and peripheral insulin resistance (Figure 5.5). Since 2004 HOMA2 calculator is widely available (<https://www.dtu.ox.ac.uk/homacalculator/>) and used in large scale studies defining insulin resistance as $\text{HOMA-IR} > 2.5$ [$(\text{Glucose}_0 \times \text{Insulin}_0)/22.5$]. Estimates from the HOMA2 correspond well ($r = -0.6$) with reference methods, such as HEC (19).

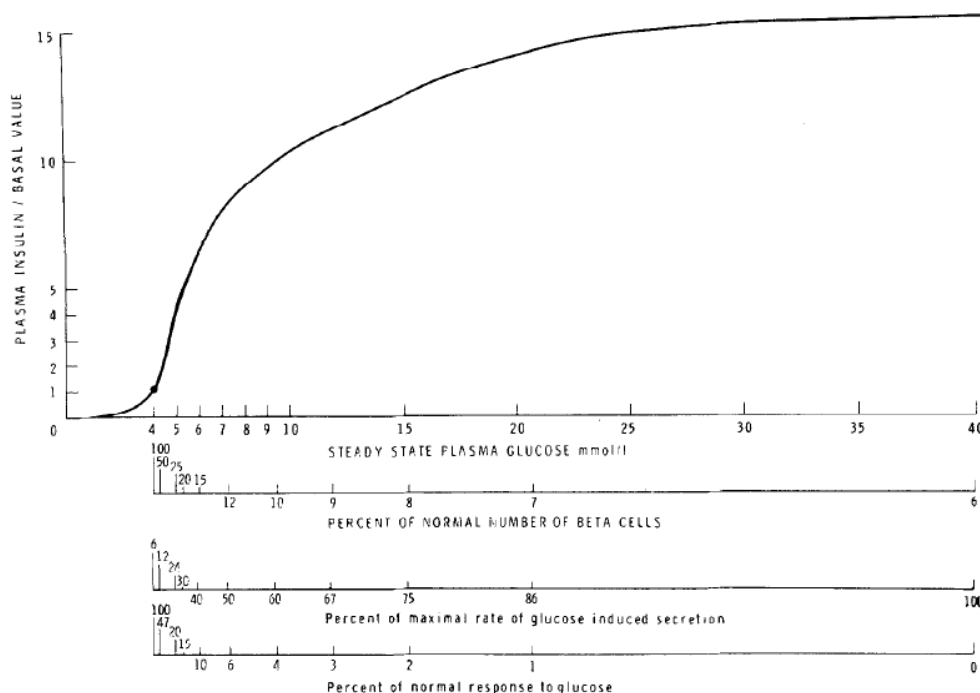


Figure 5.4: Beta cell dysfunction estimated by the insulin response. Adopted from Turner et al (1)

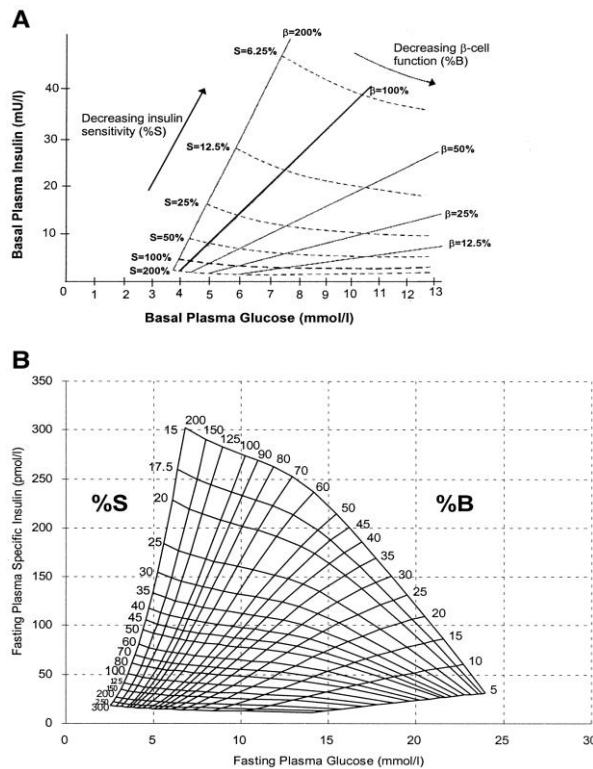


Figure 5.5: HOMA model a) presented by Turner and Holmes and b) updated by Levy et al; Adopted from Tara et al (30)

5.4.2 Matsuda Index

In 1999 Matsuda and De Fronzo (24) compared OGTT derived insulin indexes with results obtained by reference HEC. Purpose of the clamp is to suppress liver gluconeogenesis and assess peripheral insulin resistance by infusing high doses of intravenous insulin, maintaining at the same time stable glucose levels by intravenous variable rate infusion. The rate of the glucose infusion mirrors the glucose uptake by tissues, indicating insulin sensitivity. After studying 153 subjects, who underwent both HEC and OGTT, they developed a model (<http://mmatsuda.diabetes-smc.jp/2pointssi.html>) which was highly correlated ($r=0.73$) with the glucose disposal obtained from the clamp. A Matsuda Index <2.5 ($10,000/\sqrt{G_o \times I_o \times G_{mean} \times I_{mean}}$) is indicative of insulin resistance.

5.5 Statistical analysis

Data was analyzed using SPSS version 22. Statistical analysis was performed using “t test” or “Mann-Whitney U test” depending on data distribution (parametric or not). Results of the biochemical analysis were checked for potential outliers. Bivariate and partial correlations were examined between maternal, cord blood biomarkers and outcomes of

interest. A subgroup analysis was performed comparing maternal biomarkers adjusting for GDM status and ethnicity. Regression models were used to assess any association between maternal and cord biomarkers with infant body composition. Data which was not normally distributed were log transformed and the effect size was presented as “percentage change”. Results were further adjusted for potential confounders. In order to eliminate the effect of GDM treatment, association between maternal biomarkers and neonatal data was examined only in the nGDM participants. The author performed all statistical analyses. The accuracy of the methods and results were reviewed by the Chief Investigator’s senior research fellow in biostatistics.

5.6 Results

Maternal and cord blood data was available for 109 participants for all the biomarkers except HMWA. Due to an error (stop solution not added on time) during the ELISA analysis of HMWA, only 95 maternal and cord blood HMWA pairs were available for the final analysis (Figure 5.6). GDM was diagnosed in 16.5% of our participants who received either diet advice (n=6) or medical treatment with metformin or/and insulin (n=12).

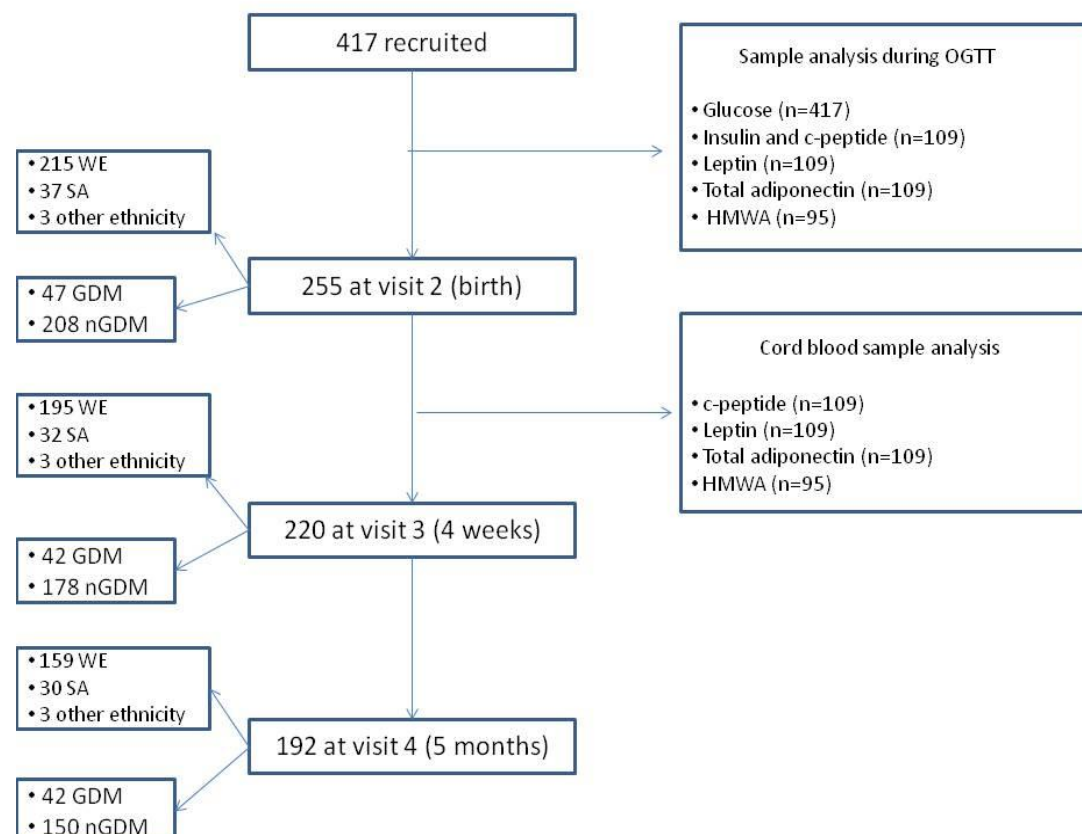


Figure 5.6: Consort diagram of available biomarkers during study visits; SA: South Asians, WE: White Europeans

5.6.1 Maternal analysis

Maternal characteristics are presented in Table 5.2. Our cohort included mothers with increased metabolic risk, fulfilling at least one of the risk factors for GDM, as indicated by NICE guidelines (31). Mean (SD) maternal BMI at 28 weeks of gestation (during OGTT) was 33.2 (6.9). After utilizing OGTT₀ and OGTT₁₂₀ glucose and insulin levels %B, HOMA-IR and Matsuda indexes were calculated. Median (IQR) maternal leptin was 32.5 (23.3, 42.8) ng/ml, mean (SD) of total and HMW adiponectin were 4.1 (1.6) µg/ml and 1.8 (0.9) µg/ml respectively.

Maternal characteristics during OGTT (n=109)	Mean (SD)
Age (yrs)	32.2 (5.1)
BMI (kg/m ²)	33.2 (6.9)
Gestational age (weeks)	27.9 (1.2)
Leptin (ng/ml)*	32.5 (23.3, 42.8)
Total adiponectin (µg/ml)	4.1 (1.6)
HMWA (µg/ml) (n=95)	1.8 (0.9)
OGTT ₀ glucose (mmol/L)	4.5 (0.4)
OGTT ₁₂₀ glucose (mmol/L)	6.0 (1.4)
%B	182.8 (51)
HOMA-IR*	2.9 (2, 4.3)
Matsuda index	4.4 (2.2)

Table 5.2: Maternal characteristics during OGTT at 28 weeks gestation. Table provides information regarding maternal adipocytokines, glucose values and insulin resistance.

*Median values with Interquartile range

Maternal BMI positively predicted glucose levels at OGTT₀ and OGTT₁₂₀. Higher levels of BMI were positively correlated with higher insulin resistance, as indicated by HOMA-IR and Matsuda indexes. Higher leptin levels were noted with raising maternal BMI ($r=0.62$, $p<0.01$). Both total ($r= -0.2$, $p=0.1$) and HMW ($r= -0.1$, $p=0.9$) adiponectin were negatively related to BMI, without reaching statistical significance (Figure 5.7, Figure 5.8, Table 5.3).

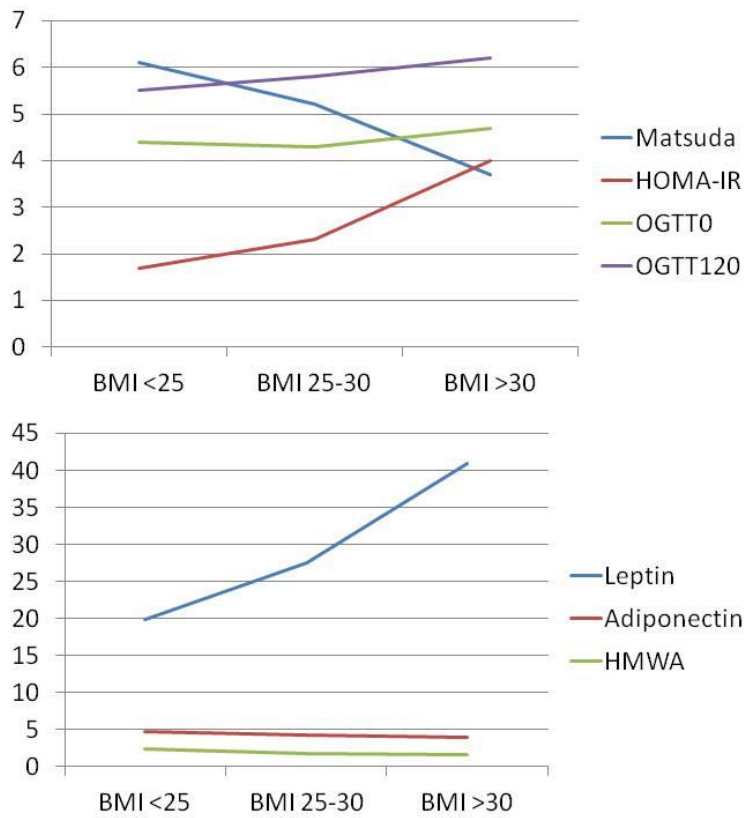


Figure 5.7: Outcomes of interest based on BMI categories. Higher BMI was correlated with higher insulin resistance, higher leptin and lower adiponectin levels

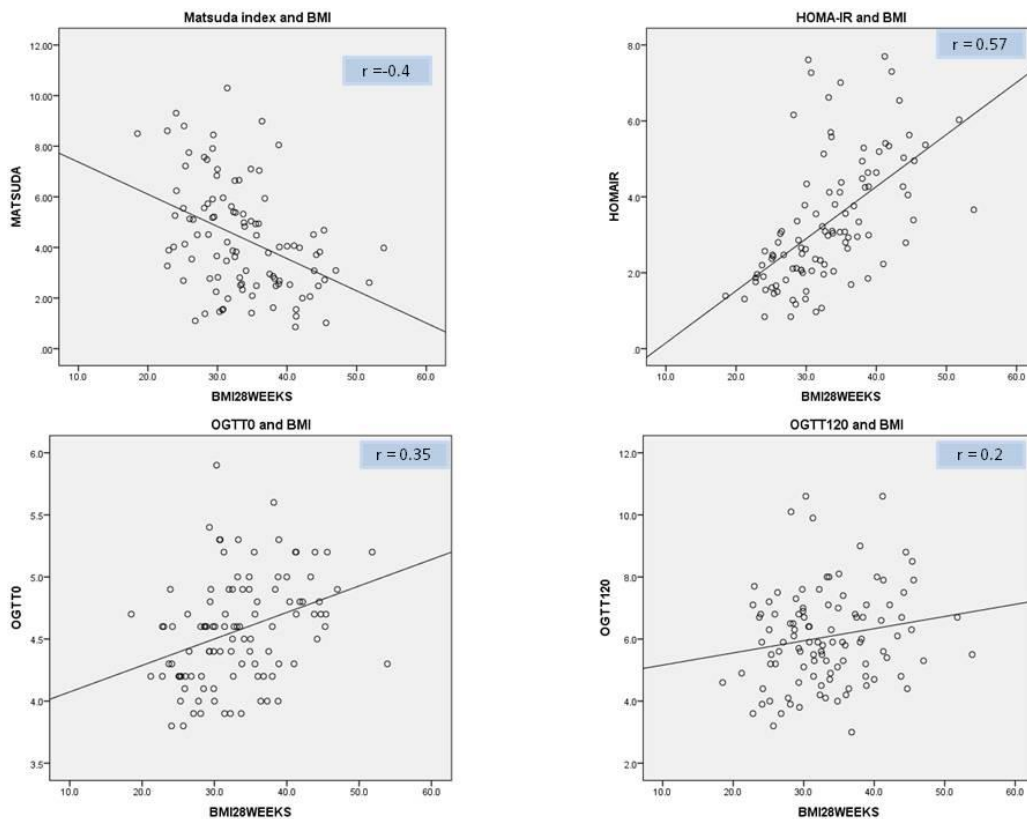


Figure 5.8: Scatter plots between Matsuda index, HOMA-IR, OGTT₀ glucose values, OGTT₁₂₀ glucose values and maternal BMI at 28 weeks gestation

	BMI at 28 weeks		
Dependent variables	β co-efficient	r value	p values
Matsuda index	-0.127	-0.40	<0.01
HOMA-IR*	1.9%	0.57	<0.01
%B	3.55	0.45	<0.01
OGTT ₀ glucose (mmol/L)	0.02	0.35	<0.01
OGTT ₁₂₀ glucose (mmol/L)	0.03	0.20	0.07
Leptin (ng/ml)*	4.4%	0.62	<0.01
Total adiponectin (μ g/ml)	-0.04	-0.15	0.1
HMWA (μ g/ml)	-0.001	-0.1	0.9

Table 5.3: Associations between maternal biomarkers and BMI at 28 weeks. BMI shows a moderate correlation with insulin resistance, pancreatic secretion and leptin levels.

There is a weak correlation with glucose values at OGTT₀ but not at OGTT₁₂₀

* Values represent percent increase (or decrease) in the response variable for every one-unit increase in the independent variable (BMI)

In order to assess the metabolic effect of maternal adipocytokines, specifically their effect on insulin metabolism, we investigated their correlations with indexes of insulin sensitivity. Increased maternal leptin was weakly associated with increased insulin resistance ($p < 0.01$). A weak negative association between both total and HMW adiponectin and insulin resistance was noted ($p < 0.05$). After adjusting for maternal BMI, all associations attenuated except HMWA which remained statistically significant. Detailed data is presented in Table 5.4. Looking at the relationship between glucose measurements during OGTT as continuous variable and maternal adipocytokines we found a weak positive correlation of maternal leptin with fasting glucose levels ($r = 0.3$, $p < 0.01$).

a)

Leptin (log.)	Unadj. β	p value	Adjusted β	p value	partial r
Matsuda*	-0.03	<0.01	-0.01	0.2	-0.2
HOMA-IR**	0.6	<0.01	0.3	0.6	0.3
%B*	0.8	<0.01	0.2	0.3	0.1
Total adip. (µg/ml)					
Matsuda	0.3	0.02	0.2	0.08	0.17
HOMA-IR***	-1.2	0.4	-0.02	0.8	-0.1
%B	-4.3	0.2	-2	0.5	-0.1
HMWA (µg/ml)					
Matsuda	0.41	0.05	0.44	0.02	0.2
HOMA-IR***	-2.4	0.4	-2.2	0.2	-0.2
%B	-10.1	0.05	-10.3	0.02	-0.3

b)

Leptin (log.)	Unadj. β	p value	Adjusted β	p value	partial r
OGTT ₀ *	0.57	<0.01	0.25	0.2	0.1
OGTT ₁₂₀ *	-0.26	0.9	-1.1	0.1	-0.1
Total adip. (µg/ml)					
OGTT ₀	-0.15	0.5	-0.002	0.9	-0.1
OGTT ₁₂₀	-0.02	0.8	0.01	0.8	0.1
HMWA (µg/ml)					
OGTT ₀	0.01	0.8	0.008	0.8	0.1
OGTT ₁₂₀	0.01	0.9	0.04	0.8	0.1

Table 5.4: Regression analysis between maternal biomarkers (independent variable) and a) insulin sensitivity/secretion indexes b) glucose values during OGTT (dependent variables); Unadjusted and adjusted for maternal BMI at 28 weeks. The correlation between maternal leptin and insulin resistance disappears after adjusting for maternal BMI

* 1% increase in the independent variable increases (or decreases) the dependent variable by x units

** Values represent percent increase (or decrease) in the dependent variable for every 1% increase in the independent variable

*** Values represent percent increase (or decrease) in the response variable for every one-unit increase in the independent variable

5.6.2 Maternal biomarkers and neonatal data

In order to eliminate the effect of GDM treatment, analysis included mothers without the diagnosis of GDM (n=91). There was no correlation between maternal and cord adipocytokines ($r=0.1$, $p>0.05$) except a weak positive correlation in the case of total adiponectin ($r=0.3$, $p=0.01$). Maternal leptin was positively correlated with infant anthropometry without reaching statistical significance. On the other hand, both maternal

total and HMW adiponectin were negatively correlated with adiposity and birthweight, but once again without reaching statistical significance (Table 5.5).

Birth		FM (kgr)	%FM	FFM (kgr)	Weight (kgr)	Cord c- peptide (pmol/lt)
Mat. Leptin* (log.)	Model 1	0.001, p=0.6	0.01, p=0.3	0.0004, p=0.8	0.001, p=0.5	19.7, p=0.8
	Model 2	p=0.6	p=0.7	p=0.2	p=0.2	p=0.8
Mat. Total adip. (µg/ml)	Model 1	-0.013, p=0.3	-0.3, p=0.2	-0.014, p=0.6	-0.02, p=0.4	0.731, p=0.9
	Model 2	p=0.5	p=0.3	p=0.9	p=0.7	-1.0, p=0.9
Mat. HMWA (µg/ml)	Model 1	-0.003, p=0.8	-0.02, p=0.9	-0.032, p=0.4	-0.034, p=0.5	3.894, p=0.8
	Model 2	p=0.4	p=0.5	p=0.3	p=0.2	-4.5, p=0.8

Table 5.5: Regression analysis between maternal adipocytokines at 28 weeks gestation and neonatal anthropometry; Model 1: unadjusted, Model 2: adjusted for maternal BMI at 28 weeks, maternal age, gestational weight gain, gestational age, gender and ethnicity
* 1% increase in the independent variable (leptin) increases the dependent variable by x units

Higher beta cell function (%B) and higher insulin resistance (HOMA-IR, Matsuda) at 28 weeks gestation were correlated with higher adiposity and birthweight. Correlations became non significant after adjusting for maternal BMI. Glucose values during OGTT₀ and OGTT₁₂₀ were positively related to neonatal FM and %FM. Maternal BMI at 28 weeks was independently related to neonatal adiposity and birthweight, even after adjusting for maternal glycaemia. Cord c-peptide was strongly correlated with maternal insulin resistance and OGTT₁₂₀ glucose levels, but not with maternal BMI or glucose levels at OGTT₀ (Table 5.6).

Birth	FM (kgr)	%FM	FFM (kgr)	Weight (kgr)	Cord c-peptide (pmol/l)
28 weeks gestation	B co-efficient (p value)				
BMI					
Model 1	0.008 (0.01)	0.157 (0.01)	0.009 (0.1)	0.017 (0.03)	0.1 (0.9)
Model 2	0.007 (0.01)	0.126 (0.02)	0.004 (0.4)	0.011 (0.1)	-0.2 (0.9)
Model 3**	0.007 (0.01)	0.125 (0.02)	0.0013 (0.06)	0.02 (0.02)	-1.5 (0.9)
OGTT₀					
Model 1	0.108 (0.01)	2.6 (0.01)	-0.09 (0.4)	0.01 (0.9)	61.1 (0.1)
Model 2	0.109 (0.01)	2.6 (0.01)	-0.1 (0.1)	0.01 (0.9)	67.1 (0.1)
Model 3	0.075 (0.1)	2.1 (0.03)	0.007 (0.2)	0.006 (0.6)	76.4 (0.1)
OGTT₁₂₀					
Model 1	0.036 (0.02)	0.86 (0.9)	0.009 (0.8)	0.04 (0.3)	31.2 (0.03)
Model 2	0.036 (0.01)	0.8 (0.03)	0.020 (0.5)	0.06 (0.1)	30.1 (0.05)
Model 3	0.032 (0.02)	0.7 (0.02)	0.004 (0.4)	0.05 (0.2)	30.3 (0.05)
Matsuda					
Model 1	-0.012 (0.2)	-0.2 (0.2)	-0.04 (0.5)	-0.05 (0.04)	-19.6 (0.02)
Model 2	-0.020 (0.01)	-0.4 (0.04)	-0.04 (0.04)	-0.06 (0.01)	-19.2 (0.03)
Model 3	-0.013 (0.09)	-0.3 (0.1)	-0.03 (0.07)	-0.05 (0.05)	-22.8 (0.01)
HOMA- IR*					
Model 1	0.002 (0.01)	0.04 (0.02)	0.001 (0.3)	0.003 (0.1)	159.3 (0.04)
Model 2	0.002 (0.01)	0.05 (0.03)	0.001 (0.09)	0.003 (0.1)	154.3 (0.06)
Model 3	0.001 (0.9)	0.04 (0.07)	0.001 (0.9)	0.002 (0.5)	253.9 (0.01)
%B					
Model 1	0.001 (0.05)	0.01 (0.2)	0.002 (0.03)	0.003 (0.02)	0.5 (0.1)

Table 5.6: Correlations between maternal characteristics at 28 weeks gestation, neonatal body composition and cord c-peptide. Maternal BMI and glucose levels independently predict neonatal adiposity. Insulin resistance, but not BMI, predict cord c-peptide levels Model 1: unadjusted, Model 2: adjusted for gestational weight gain, maternal age, gestational age, gender and ethnicity, Model 3: Model 2 + maternal BMI at 28 weeks gestation;

* 1% increase in the independent variable increases (or decreases) the dependent variable by (coefficient/100) units;

** Model 2 + glucose levels at OGTT₀ and OGTT₁₂₀

5.6.3 Cord adipocytokines, cord c-peptide and body composition at birth

Cord c-peptide and body composition were available for 109 newborns. Mean (SD) BW was 3.368 (0.510) kgr and mean (SD) %FM was 11.3 (4.0) %. Median (IQR) cord leptin level was 7.3 (4.2, 11.8) ng/ml. Mean (SD) total and HMW adiponectin levels were 19.3 (8.5) µg/ml and 10.7 (5.2) µg/ml respectively. Females were found to have numerically higher leptin [7.9 (4.6, 12.3) vs. 5.8 (3.1, 11.0) ng/ml, $p=0.1$] and adiponectin levels (19.5 ± 9.6 vs. 18.9 ± 8.8 µg/ml, $p=0.7$). Mean (SD) cord c-peptide levels were 500 (185) pmol/l. Cord c-peptide was moderately related with all cord adipocytokines ($r=0.3$, $p<0.01$). Table 5.7 shows correlations between cord adipocytokines, cord c-peptide and measures of neonatal body composition. 1% increase in cord leptin levels were related to 0.04% increase in %FM and 0.006 kgr increase in BW. Similarly both total and HMW adiponectin were positively related with neonatal adiposity. Adjustments for maternal characteristics and gender did not alter the results. Partial correlation (r) for %FM was 0.3 for cord leptin, 0.2 for total and 0.3 for HMW adiponectin. Cord c-peptide levels were positively correlated with neonatal anthropometry without reaching significance and they revealed moderate positive correlation with cord adipocytokines ($r=0.4$, $p<0.01$)

Cord samples	FM	%FM	FFM	BW
Leptin (log)*	B co-efficient (p value)			
Model 1	0.003 (0.01)	0.04 (0.01)	0.004 (0.01)	0.006 (0.01)
Model 2	0.002 (0.01)	0.03 (0.01)	0.003 (0.01)	0.005 (0.01)
Total adip. (µg/ml)				
Model 1	0.005 (0.01)	0.1 (0.01)	0.004 (0.4)	0.005 (0.01)
Model 2	0.004 (0.03)	0.08 (0.03)	0.003 (0.4)	0.003 (0.5)
HMWA (µg/ml)				
Model 1	0.009 (0.01)	0.2 (0.01)	0.006 (0.4)	0.015 (0.1)
Model 2	0.009 (0.01)	0.18 (0.01)	0.005 (0.6)	0.013 (0.1)
C-peptide (pmol/l)				
Model 1	0.0001 (0.1)	0.003 (0.3)	0.0001 (0.1)	0.0001 (0.08)
Model 2	0.0001 (0.2)	0.002 (0.5)	0.0001 (0.09)	0.0001 (0.09)

Table 5.7: Correlations between cord adipocytokines and neonatal body composition. Data suggests a weak correlation between cord adipocytokines and neonatal adiposity. Model 1: unadjusted, Model 2: adjusted for gestational age, maternal BMI at 28 weeks, gestational weight gain, gender and ethnicity

*1% increase in the independent variable increases the dependent variable by x units

5.6.4 Cord adipocytokines and body composition at Visit 3 (4 weeks)

Mean (SD) neonatal age at visit 3 assessment was 34 (6) days. Cord adipocytokines and c-peptide remained positively correlated with measures of adiposity, weight and weight gain since birth, but p value was not reaching statistical significance. Adjustments for maternal characteristics, gender and feeding mode did not alter the results (Table 5.8).

Cord samples	FM	%FM	FFM	Weight	Weight gain
Leptin (log)*	B co-efficient (p value)				
Model 1	0.002 (0.02)	0.02 (0.1)	0.005 (0.01)	0.007 (0.01)	0.001 (0.2)
Model 2	0.001 (0.2)	0.01 (0.6)	0.004 (0.01)	0.005 (0.01)	0.001 (0.7)
Total adip. (µg/ml)					
Model 1	0.004 (0.2)	0.06 (0.2)	0.002 (0.7)	0.005 (0.4)	0.001 (0.8)
Model 2	0.00 (0.2)	0.06 (0.2)	0.002 (0.7)	0.005 (0.4)	0.002 (0.6)
HMWA (µg/ml)					
Model 1	0.005 (0.3)	0.06 (0.5)	0.009 (0.3)	0.014 (0.2)	-0.001 (0.9)
Model 2	0.007 (0.2)	0.08 (0.3)	0.009 (0.2)	0.016 (0.15)	0.003 (0.7)
Cord c-peptide (pmol/l)					
Model 1	0.0001 (0.2)	0.003(0.2)	0.001 (0.5)	0.001 (0.9)	0.0001 (0.2)
Model 2	0.0001 (0.5)	0.001(0.7)	0.001 (0.5)	0.001 (0.8)	

Table 5.8: Correlations between cord adipocytokines, cord c-peptide and neonatal body composition at visit 3 (4 weeks). Cord biomarkers are not associated with body composition 4 weeks post partum;

Model 1: unadjusted, Model 2: adjusted for maternal age, gestational age, maternal BMI at 28 weeks, gestational weight gain, gender, ethnicity and feeding mode

*1% increase in the independent variable increases the dependent variable by x units

5.6.5 Cord adipocytokines and body composition at Visit 4 (5 months)

Mean (SD) neonatal age at visit 4 assessment was 161 (17) days. There was a shift from positive to negative correlation between cord leptin, cord c-peptide and infant anthropometry at 5 months post partum (Figure 5.9). After adjusting for covariates the correlation between cord leptin and %FM became significant ($p=0.03$). We were unable to demonstrate any association between total, HMW adiponectin and infant anthropometry at visit 5. All cord adipocytokines were negatively correlated with weight gain from birth to 5 months of age, with c-peptide reaching statistical significance (Table 5.9). Subgroup analysis excluding cases with GDM did not alter the results.

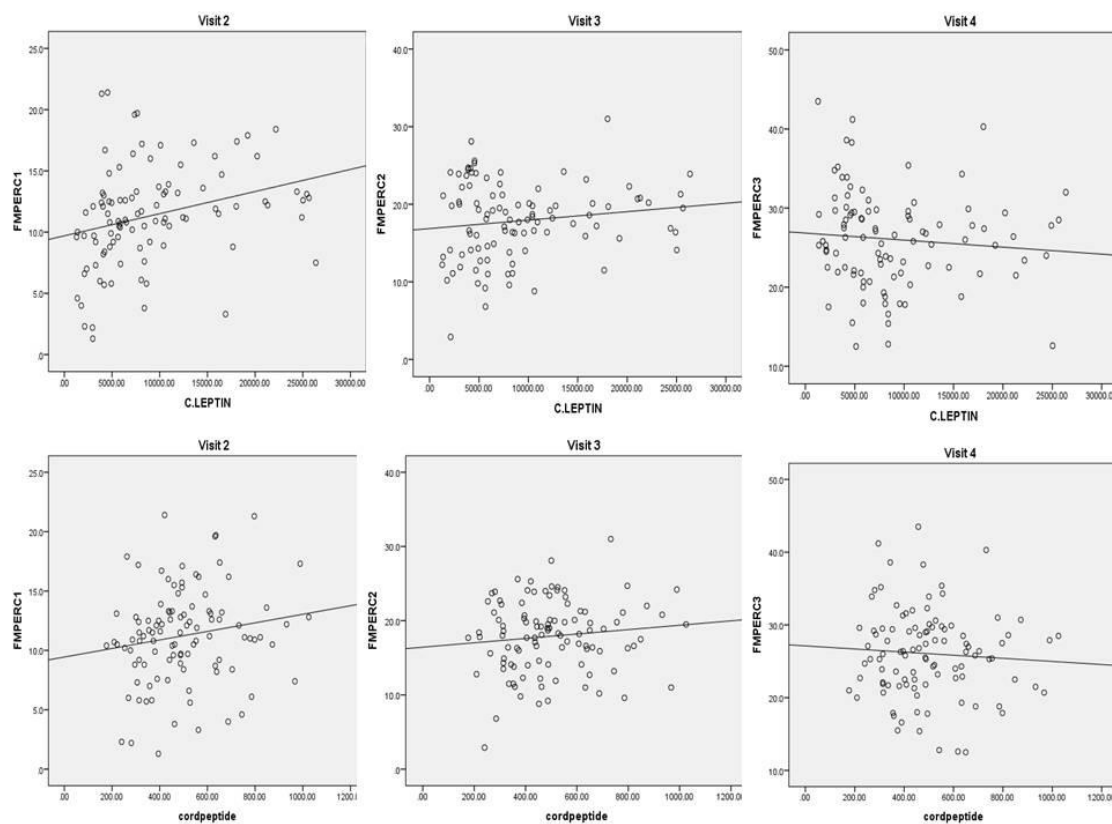


Figure 5.9: Trends between cord leptin, cord c-peptide and percentage fat mass (%FM) at birth and follow up visits. At visit 4 (5 months) relationship between cord biomarkers and %FM reverses from positive to negative

Cord samples	FM	%FM	FFM	Weight	Weight gain
Leptin (log)*	B co-efficient (p value)				
Model 1	-0.002 (0.3)	-0.03 (0.1)	0.003 (0.08)	0.001 (0.6)	-0.004 (0.1)
Model 2	-0.003 (0.1)	-0.04 (0.03)	0.004 (0.05)	0.001 (0.8)	-0.004 (0.2)
Total adip. (µg/ml)					
Model 1	-0.001 (0.9)	-0.007 (0.9)	0.002 (0.7)	0.0002 (0.8)	-0.002 (0.8)
Model 2	0.001 (0.8)	0.002 (0.9)	0.002 (0.7)	0.004 (0.7)	-0.001 (0.9)
HMWA (µg/ml)					
Model 1	0.004 (0.7)	-0.01 (0.9)	0.012 (0.3)	0.014 (0.4)	-0.001(0.9)
Model 2	0.007 (0.5)	0.02 (0.8)	0.008 (0.4)	0.015 (0.4)	0.001 (0.9)
C-peptide(pmol/l)					
Model 1	-0.0001 (0.2)	-0.002 (0.5)	-0.0001 (0.3)	-0.001 (0.1)	-0.001 (0.03)
Model 2	-0.001 (0.1)	-0.005 (0.1)	-0.0001 (0.6)	-0.001 (0.1)	-0.001 (0.03)

Table 5.9: Correlations between cord adipocytokines, cord c-peptide and neonatal body composition at visit 4 (5 months). Cord leptin is correlated negatively with adiposity whereas adiponectin seems to have no predictive value in body composition evolution in the first 5 months of life;

Model 1: unadjusted, Model 2: adjusted for gestational age, maternal BMI at 28 weeks, gestational weight gain, gender, ethnicity and feeding mode

*1% increase in the independent variable increases the dependent variable by x units

5.6.6 Metabolic biomarkers, GDM and ethnicity

We performed a subgroup analysis looking at maternal biomarkers' patterns at 28 weeks of gestation after adjusting for GDM and ethnicity (Table 5.10). Participants with GDM (n=18) were found to have higher insulin resistance (HOMA-IR, Matsuda) and higher beta cell function when compared to the nGDM group (n=88). Serum leptin was noted to be significantly higher in the GDM group after adjusting for maternal BMI, age, gestational age and ethnicity (33.8 vs 32.5 ng/ml, p=0.05). On the other hand, there was no statistically significant difference between the groups for serum total and HMW adiponectin, with the GDM group though having consistently numerically lower levels (total: 3.9 vs. 4.2 µg/ml, p=0.8; HMWA: 1.8 vs. 2 µg/ml, p=0.3, Figure 5.10).

At birth, offspring of GDM pregnancies had lower weight (3.201±0.288 vs. 3.407±0.538 kgr, p=0.1), driven predominantly by lower FFM (2.820±0.205 vs. 3.011±0.419 kgr, p=0.06). Cord levels of leptin, adiponectin and HMWA were similar between the two groups. Despite no differences in measures of adiposity, offspring of GDM pregnancies were found to have higher c-peptide levels (610±245 vs. 478±164 pmol/l, p=0.007). After adjusting for maternal and neonatal characteristics differences in c-peptide levels

remained significant. Addition of insulin resistance indexes (Matsuda, HOMA-IR) to the regression models attenuated the results (Table 5.11, Figure 5.11).

We further assessed differences between South Asian (SA, n=15) and White European (n=87) population. After adjusting for confounders (including GDM status), markers of beta cell function and insulin resistance were similar between the two groups. There was no difference in maternal serum adipocytokines (Table 5.10, Figure 5.10).

Neonatal data revealed that offspring of SA origin were lighter at birth (3.257 ± 0.454 vs. 3.405 ± 0.504 kgr, $p=0.2$), with lower FM (0.302 ± 0.148 vs. 0.411 ± 0.176 kgr, $p=0.02$) and %FM ($9.0 \pm 3.9\%$ vs. 11.7 ± 3.9 , $p=0.01$). There was no difference in cord leptin and c-peptide levels between the two groups, but the SA group was found to have lower adiponectin and HMWA. Results remained unchanged after adjusting for maternal and neonatal characteristics (Table 5.12).

a)

During OGTT	GDM (n=18)	nGDM (n=88)	p value	adjusted p
Matsuda	2.5 (1.2)	4.8 (2.1)	<0.01	<0.01
HOMA-IR*	4.9 (3.5, 5.4)	2.7 (1.8, 3.7)	<0.01	<0.01
%B	199 (69)	181 (51)	0.2	0.8
Leptin (ng/ml)*	33.8 (22.8, 47.6)	32.5 (24.6, 42.8)	0.9	0.05
Total adip. (µg/ml)	3.9 (1.6)	4.2 (1.6)	0.5	0.8
HMWA (µg/ml)	1.8 (0.9)	2.0 (1.9)	0.5	0.3

b)

During OGTT	SA (n=15)	White Europeans (n=87)	p value	adjusted p
Matsuda	4.5 (2.0)	4.3 (2.1)	0.7	0.2
HOMA-IR*	2.4 (2, 3)	3 (2, 4.6)	0.1	0.2
%B	175 (34)	186 (58)	0.4	0.3
Leptin (ng/ml)*	29.8 (20.4, 38.2)	32.5 (23.3, 46.4)	0.3	0.09
Total adip. (µg/ml)	4.3 (2.1)	4.1 (1.5)	0.6	0.9
HMWA (µg/ml)	1.7 (1.0)	1.8 (0.9)	0.6	0.6

Table 5.10: Comparison of maternal biomarkers based on a) GDM status b) ethnicity. Mothers with GDM have higher insulin resistance and leptin levels. Subgroup analysis based on ethnic background did not reveal any differences; data represent mean (SD) values

* Data is expressed as median (IQR)

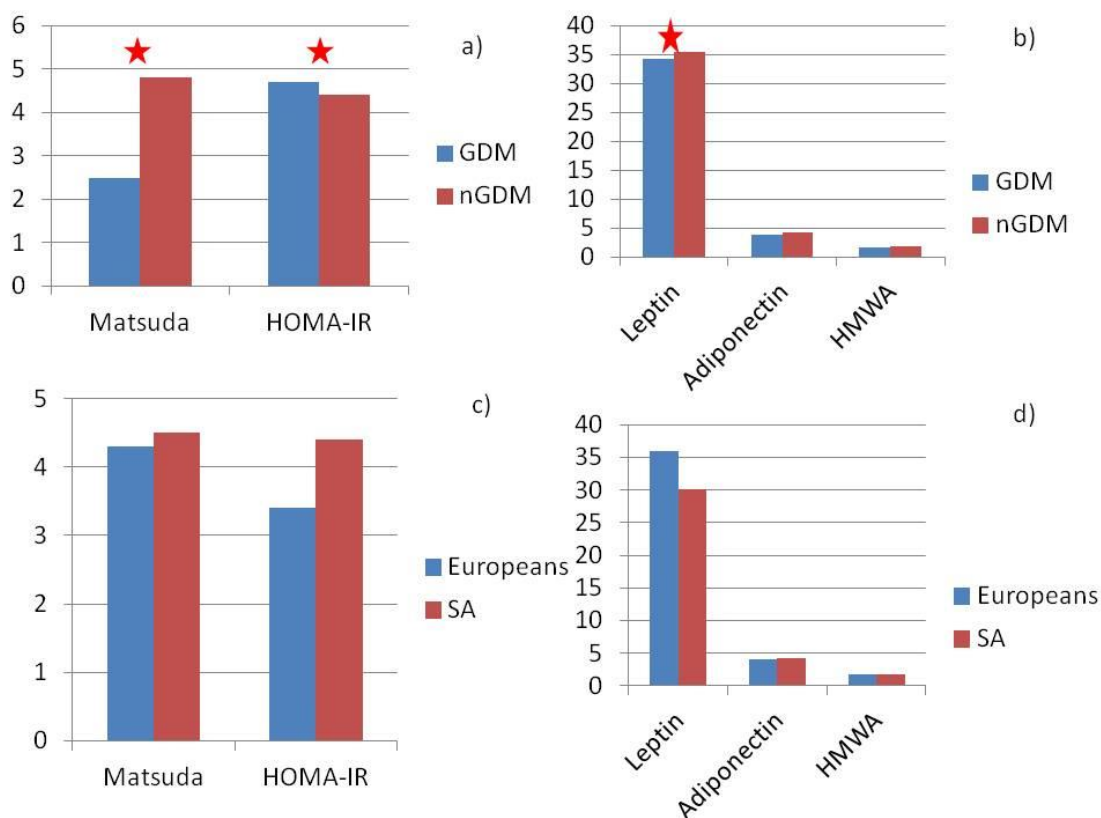


Figure 5.10: Schematic representation of differences in maternal biomarkers based on a, b) GDM status and c, d) ethnicity. Asterisk suggests statistically significant difference

a)

	GDM (n=18)	nGDM (n=88)	p value
Birthweight (kgr)	3.201 (0.288)	3.407 (0.538)	0.1
%FM	11.6 (4.3)	11.2 (3.9)	0.7
FM (kgr)	0.380 (0.160)	0.396 (0.181)	0.7
FFM (kgr)	2.820 (0.205)	3.011 (0.419)	0.06
Leptin (ng/ml)	10.8 (8.2)	8.8 (6.1)	0.2
Total adip. (µg/ml)	21.2 (13.1)	18.8 (8.2)	0.3
HMWA (µg/ml)	10.9 (6.8)	10.8 (5.4)	0.9
c-peptide (pmol/l)	610 (245)	478 (164)	0.007

b)

Cord c-peptide (pmol/l)	β co-efficient	p value
Model 1	140	0.005
Model 2	150	0.003
Model 3	110	0.03

Table 5.11: a) Comparison of anthropometric measurements and cord blood biomarkers between offspring of GDM and nGDM pregnancies b) Regression analysis of cord c-peptide levels. Infants of GDM pregnancies have higher cord peptide levels even after adjusting for maternal characteristics and infant anthropometry ; Model 1: adjusted for gender, birthweight, %FM, FFM, gestational age; Model 2: model 1 + booking maternal BMI and gestational weight gain; Model 3: model 2 + Matsuda index

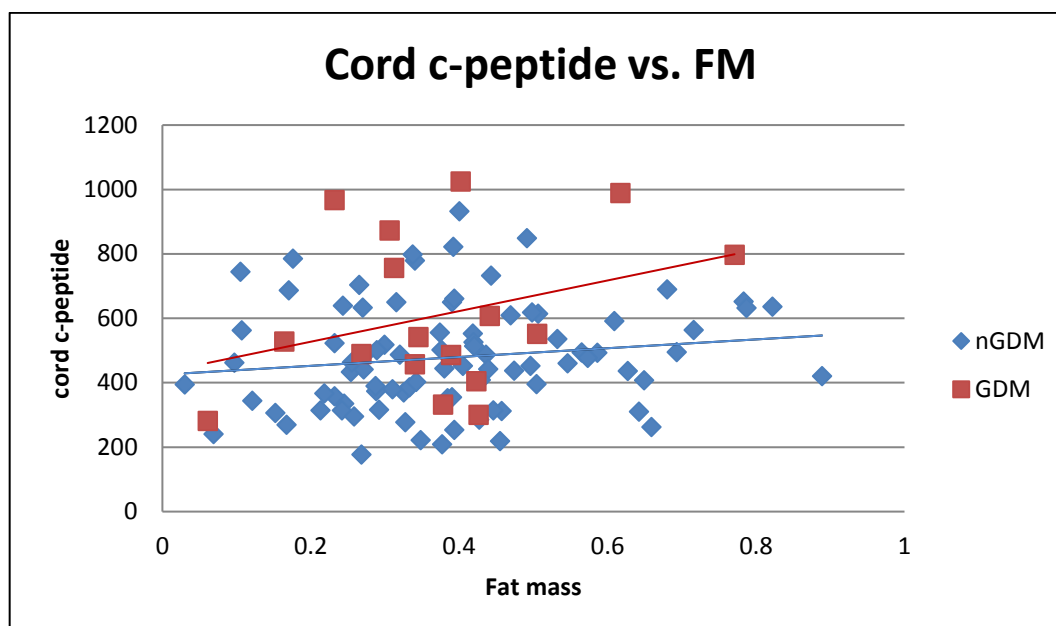


Figure 5.11: Association between cord c-peptide and fat mass (FM) in offspring of GDM and nGDM pregnancies. The GDM group has higher c-peptide levels for a given FM value

	SA (n=15)	White Europeans (n=87)	p value
Birthweight (kgr)	3.257 (0.454)	3.405 (0.504)	0.2
%FM	9.0 (3.9)	11.7(3.9)	0.01
FM (kgr)	0.302 (0.148)	0.411 (0.176)	0.02
FFM (kgr)	2.954 (0.374)	2.994 (0.391)	0.7
Leptin (ng/ml)	8.3 (5.6)	9.3 (6.6)	0.6
Total adip. (µg/ml)	14.4 (7.6)	20.1 (9.2)	0.02
HMWA (µg/ml)	6.7 (3.5)	11.6 (5.6)	0.03
c-peptide (pmol/l)	470 (182)	506 (186)	0.5

Table 5.12: Comparison of anthropometric measurements and cord blood biomarkers between offspring of SA and White European descent. SA infants have lower leptin (numerically) and adiponectin levels, driven by lower FM. There are no signs of early insulin resistance in the SA group, as indicated by the proportional c-peptide levels

5.7 Discussion

Our study reveals five key findings: i) Increased insulin resistance, characterizing maternal obesity and GDM, is positively related to increased fetal adiposity and mediated by higher cord c-peptide levels; ii) Offspring of GDM pregnancies have higher c-peptide levels for a given FM, which is suggestive of early development of insulin resistance; iii) Maternal adipocytokines are related to insulin resistance but not to cord adipocytokines

and neonatal adiposity; iv) Maternal glycaemia in the sub diabetic range and maternal BMI independently predict neonatal adiposity and v) Cord blood adipocytokines are markers of neonatal adiposity, with cord leptin and c-peptide levels negatively predicting adiposity and weight gain at 5 months of age.

5.7.1 Maternal insulin resistance: “the missing link”

Maternal BMI and GDM are factors contributing to the developmental programming of obesity and metabolic disease (32), with adverse phenotype being present at birth (6, 33). Maternal obesity is characterized by higher fasting and postprandial glucose levels (4) raising the question of whether the association between hyperglycaemia and neonatal fat mass is due to BMI rather than hyperglycaemia per se. Performing a subgroup analysis in the nGDM group in an attempt to eliminate the effect of GDM treatment, we showed that maternal glycaemia in the sub diabetes range during OGTT (OGTT₀ and OGTT₁₂₀) and maternal obesity independently predict neonatal adiposity. Regarding maternal hyperglycaemia, we observed a stronger effect size of glucose levels at OGTT₀ on neonatal %FM (β co-efficient: OGTT₀= 2.1 %FM vs. OGTT₁₂₀=0.7 %FM). Using surrogate markers of insulin sensitivity (HOMA-IR, Matsuda) we showed that insulin resistance is the common denominator for both conditions, leading to hyperfunctioning of beta cells (raised %B) in order to overcome the resistance and maintain euglycaemia. Furthermore, we demonstrated that higher insulin resistance is linked to higher neonatal hyperinsulinaemia (indicated by cord c-peptide levels), increased neonatal adiposity and birthweight.

In an attempt to investigate the missing link between maternal intrauterine environment and neonatal body composition we looked at associations between maternal metabolic profile and cord blood biomarkers. Of great interest is the fact that despite the correlation between insulin resistance and fetal hyperinsulinaemia we were unable to demonstrate any direct association between maternal BMI, glucose levels during OGTT and cord c-peptide, except a borderline correlation with OGTT₁₂₀ values (β =30.3, p =0.05). Given the fact that maternal insulin does not cross the placenta (34) the above observation can be explained by the “fuel mediated teratogenesis” hypothesis developed by Freinkel (35). According to this, a variety of nutrients in addition to glucose may be transported from maternal to fetal circulation across the placenta, stimulating fetal insulin production and leading to increased adiposity. Insulin resistance is related to raised serum glucose, triglycerides, free fatty acids and amino acids levels in maternal circulation. The excessive nutrient transport to fetal circulation results in hyperinsulinaemia, macrosomia

and adiposity (36). Studies from Schaefer et al (7) and Di Cianni et al (37) have supported the above hypothesis by showing that maternal lipids and amino acids are linked to macrosomia independent of maternal glucose levels and BMI. In our cohort, cord c-peptide levels were positively related to neonatal fat mass and weight, albeit not reaching statistical significance. We speculate that this is due to sample size and a clinically important correlation between fetal hyperinsulinaemia and adiposity at birth really exists. Evidence from the HAPO study supports our theory (3). After examining the association between 19,885 cord c-peptide samples and neonatal anthropometry they suggested an approximate effect size of 0.4gr in birthweight and 0.003% in %FM for every 1pmol/L increase in cord c-peptide levels. Results of our cohort are very similar suggesting an effect size of 0.1 gr for birthweight and 0.002% for %FM. Overall, we report that the effect of adverse intrauterine environment on neonatal body composition is mediated by increased fetal hyperinsulinaemia.

5.7.2 Development of early insulin resistance

Treatment of GDM is known to reduce perinatal adverse outcomes (38) but not the risk of future obesity (39). Catalano et al (40) has shown that pregravid BMI is related to increased fetal insulin resistance even after adjusting for neonatal FM. Consistent with these findings, Luo et al (41) proved that insulin resistance during OGTT is related to increased cord insulin and reduced fetal glucose to insulin ratio. The above results suggest that insulin resistance may be present even at birth in pregnancies complicated by maternal adverse outcomes. Our study is the first to assess the association between maternal and neonatal biomarkers with offspring's body composition obtained by objective measures of adiposity. We reveal that optimal glycaemic control in pregnancies complicated by GDM reduces neonatal macrosomia and adiposity but fails to improve offspring's metabolic trajectories. We report that offspring of pregnancies complicated by GDM, despite maternal glucose optimization, have higher cord c-peptide levels for a given FM compared to the nGDM group, suggesting the presence of in utero beta-cell dysfunction and/or insulin resistance. Fetal exposure to adverse maternal intrauterine environment before the diagnosis of GDM seems to alter structural and functional development of organs leading to early life insulin resistance. It is interesting to note that recent HAPO follow-up study showed that maternal fasting and post-load glucose levels at OGTT predicts offspring fasting and post-glucose levels, respectively (42).

Cord blood glucose levels were not available in our study therefore we used cord c-peptide levels for a given amount of FM as a proxy for fetal insulin resistance. Maternal

insulin does not cross the placenta (43) and fetal insulin is the main growth and anabolic factor during intrauterine development, favoring fat to fat free mass deposition (44). However, cord blood c-peptide levels except pancreatic insulin secretion may also reflect insulin sensitivity (8). One of the main limitations of this approach is that administration of intravenous glucose (in cases of GDM pregnancies) during delivery could potentially bias the results by transiently increasing fetal insulin secretion. Although we recognize the above limitation we speculate that it is unlikely to contribute to our findings. In our study, all pregnancies were delivered at the Delivery Suite of George Eliot Hospital, in Nuneaton, UK. Trust guidelines dictate that all pregnancies complicated by GDM should have a very tight glucose control during delivery, achieved by the implementation of variable rate insulin infusion (VRII). Purpose of the protocol is to maintain maternal glucose levels between 4-7.8 mmol/lit by simultaneous infusion of glucose solutions and insulin.

5.7.3 The role of leptin and adiponectin

Adipose tissue has been recognized as an endocrine organ contributing to the complexity of the “obesity genesis” (45). The most biological active and well described adipocytokines are leptin and adiponectin. Leptin inhibits the anabolic effect of insulin, causing reduced lipogenesis, reduced glycogenesis and increased fattyacid oxidation (46). Raised leptin levels has a direct, suppressive effect of pancreatic insulin production (47). Obesity is characterized by insulin and leptin resistance, with levels being in proportion with BMI and visceral adiposity (48). Our results are in agreement with the above literature. We found that maternal BMI is positively related to leptin levels during pregnancy. We also demonstrated that higher leptin levels are related to higher insulin resistance. The presence of leptin resistance is confirmed by the positive correlation between leptin and beta cell secretory function, indicated by %B. We were unable to demonstrate any correlation between maternal leptin levels and cord leptin levels or neonatal anthropometry. Our findings confirm the “two compartment model” between maternal and fetal circulation suggesting that maternal leptin cannot cross the placenta due to its high molecular weight (49).

Adiponectin has a pivotal role in energy homeostasis, inflammation and cell proliferation. Low levels are observed in cases of obesity, insulin resistance, type 2 diabetes mellitus and GDM. The HMW isoform has been shown to be more metabolically active and more closely related to insulin sensitivity when compared to total adiponectin (13). In our cohort, maternal BMI and GDM were inversely related to adiponectin levels. We have

shown a negative correlation with insulin resistance but not with glucose levels during OGTT. After assessing indexes of maternal metabolic health we were unable to demonstrate any superiority of HMW over total adiponectin. In contrast to our results, Fisher et al (50) showed that in Indo-Asian males (n=34), adiponectin was negatively correlated with OGTT₁₂₀ values (stronger association with HMWA). Hara et al (51) showed that both total and HMWA are related to insulin resistance in Japan adult population (n=171) but after adjusting for maternal BMI and gender only HMWA remained significant. Ethnic variability and small sample size are the most likely contributing factors for these discordant results. Furthermore, the physiology of adiponectin is different during pregnancy and affected by placental signaling (fetal adiponectin levels reveal the nutritional status of the fetus through placental signaling), thus direct comparison may not be reliable (52). Adiponectin values in serum and adipose tissue drop after mid pregnancy in an attempt to promote nutrient mobilization to the growing fetus (53).

Following the results of our systematic review we tried to assess the predictive value of cord blood biomarkers in neonatal adiposity up to 5 months of age. One of the main strengths of our study was the use of objective measure of adiposity (PEAPOD) to calculate body composition. Mean cord blood adiponectin (19.3 µg/ml) was almost five times higher compared to maternal levels (4.18 µg/ml), findings consistent with current literature (54). Although our mean cord blood leptin level was higher, the median levels in our study (7.3ng/ml) were similar to the results of our systematic review (mean cord leptin = 9.1ng/ml) and to those presented by Karakosta et al (mean cord leptin = 7.7 ng/ml). Our results reveal that cord leptin and adiponectin are positively associated with adiposity at birth, suggesting these are useful objective markers for adiposity. The association between cord leptin, infant FM and weight gain remained positive at 4 weeks but shifted to negative at 5 months post partum. This was similar to what was seen in our systematic review. As already extensively described in chapter 2, we speculate that infants with higher leptin levels develop a 'compensatory behavior' driven by the anorexigenic effect of leptin in early postnatal life. In order to maintain positive energy balance ensuring adequate brain and visceral organ development leptin's full metabolic effect is not exerted until two weeks post partum (55), observation which could potentially explain the positive correlation with body composition at 4 weeks. We were unable to show any consistent patterns between cord adiponectin levels and body composition during the first months postpartum.

5.7.4 The role of cord c-peptide

One of the notable findings of our analysis is the correlation between cord c-peptide and body composition in the early post natal life. C-peptide seems to parallel leptin, having a positive correlation with adiposity in the first 4 weeks, shifting to negative at 5 months of age. The effects of fetal and neonatal insulin on hypothalamic development have not been extensively studied in vivo due to the hypoglycaemia cross effect. In humans, hypothalamic development occurs during gestation and early post natal life. One of the most important components of hypothalamus is the arcuate nucleus (ARC), playing an important role in energy homeostasis and appetite regulation. Projections from ARC develop during pregnancy with further refinements occurring in the early post natal period (56). Similarly to leptin, data from neuronal cell lines suggest that insulin has a neurotrophic action participating in neurite outgrowth development (57). Perinatal hyperinsulinaemia may be a programming factor causing malformation of hypothalamic structures and future obesity. Animal studies showed that early post natal insulin administration leads to increased hyperphagia, obesity and insulin resistance in adulthood (58, 59). Blockage of central insulin signal in genetically modified rodents exposed to high maternal diet led to restoration of ARC projections and normalization of metabolic profile (60). In cases of adverse intrauterine environment, insulin seems to be one of the metabolic hormones affecting hypothalamic development (Figure 5.12) (61). Maternal diabetes and obesity, resulting in fetal hyperinsulinaemia, have been shown to impact on the structure of the ARC by promoting orexigenic to anorexigenic neuron formation (62). We speculate that the negative correlation with infant adiposity observed at 5 months is predominantly driven by higher leptin levels rather than a direct effect of c-peptide per se, as cord c-peptide and leptin levels are weakly-positively correlated ($r=0.35$, $p=0.01$). It would be of great interest to follow this cohort in the future to investigate whether cord c-peptide can predict future adiposity through adverse hypothalamic programming.

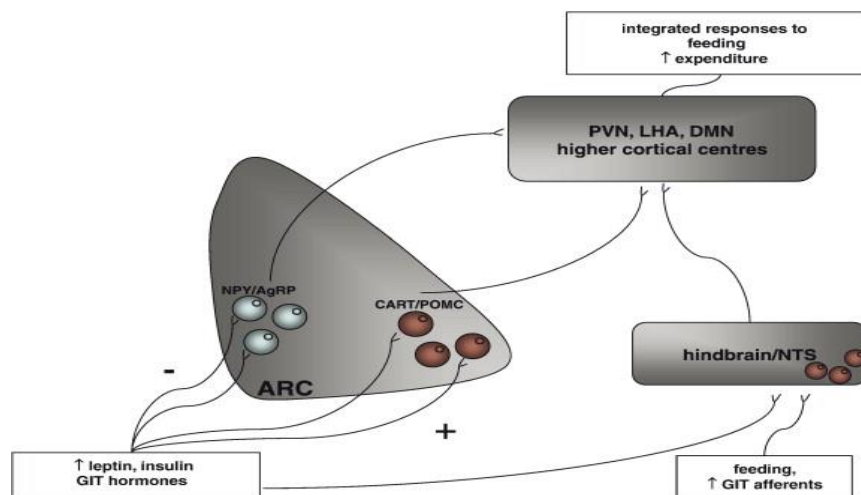


Figure 5.12: Role of metabolic hormones in the development of hypothalamic circuitry; ARC: arcuate nucleus; GIT: gastrointestinal tract; PVN: paraventricular nucleus; LHA: lateral hypothalamic area; DMH: dorsomedial nucleus; NTS: nucleus of solitary tract; Adopted from Cottrell et al (63)

Limitation of our study is the fact that cord HMWA levels were not available for 14 neonates due to laboratory error during ELISA analysis. The main strength of our study is the use of PEAPOD, a direct method of assessing body composition, instead of fat mass equations derived by skinfold thickness measurements. Our study, despite the relative small number, shares common characteristics and findings with the landmark HAPO trial (Table 5.13) (3). Basic demographics are common between the two groups and both results support the theory that maternal hyperglycaemia and BMI independently predict neonatal adiposity, a relationship mediated by fetal hyperinsulinaemia. The discordance between %FM and cord c-peptide in the two studies can be attributed to the different methods of assessing neonatal adiposity (PEAPOD vs. FM equations).

	PEAPOD study (n=109)	HAPO (n=19,885)
Maternal BMI (kgr/m²)	32.6 (6)	27.5 (5)
OGTT₀ (mmol/l)	4.5 (0.4)	4.5 (0.4)
OGTT₁₂₀ (mmol/l)	6.0 (1.4)	6.2 (1.3)
Gestational age (weeks)	39.2 (1.4)	39.4 (1.6)
Birthweight (kgr)	3.368 (0.510)	3.308 (0.505)
%FM at birth	11.3 (4)	11.3 (3.7)
Cord c-peptide (pmol/l)	500 (185)	331 (198)

Table 5.13: Comparison of maternal and neonatal characteristics between our study (PEAPOD) and HAPO. Our cohort included high risk pregnancies with higher maternal BMI. Despite similar FM at birth, cord c-peptide levels were discordant. HAPO study used anthropometric indexed to derive infant adiposity; Data represent mean (SD) values

In conclusion, we showed that maternal hyperglycaemia in the sub diabetes range and maternal obesity independently predict neonatal adiposity, a correlation mediated by fetal hyperinsulinaemia. We demonstrated that despite optimal glycaemic control, offspring of pregnancies complicated by GDM are metabolically compromised at birth, suggesting that adverse metabolic programming has already taken place by the time of GDM diagnosis. We also revealed that cord leptin and c-peptide may be useful markers in predicting future adiposity by adverse intrauterine programming, thus allowing early identification of high risk groups with a single blood test. Follow up studies of our cohort with body composition assessment in childhood and adolescence is crucial in order to understand the long term effects of maternal metabolic environment and the predictive value of cord blood biomarkers in future obesity. If prevention is the key to tackle the obesity epidemic, preconception and early postpartum periods may be an important focus of future research.

5.8 References

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Chapter 6

Comparison of fat mass equations and anthropometric indexes with reference criterion (PEAPOD)

6.1 Introduction

Birthweight and BMI have been widely used to identify risk of future obesity and metabolic diseases. Evidence suggests a “U” shaped association between weight at birth and future obesity (1), with low birthweight having a stronger effect when compared to macrosomia (2). BMI is derived from two simple anthropometric measurements (weight, height) allowing for precision, accuracy and repeatability of the results. Higher BMI values are related to higher prevalence of type 2 diabetes mellitus, hypertension and dyslipidaemia, traced from childhood to adulthood (3, 4). Both birthweight and BMI are poor indicators of nutrition and growth as they are unable to differentiate between FM and FFM. BMI changes during childhood are more likely to reflect changes in FFM rather than FM (5). Furthermore, the relationship between BMI or weight and percentage of FM differs between various populations. Asian adults are known to have up to 5% higher FM for a given BMI when compared to White Europeans (6).

In previous chapters, we have extensively described the role of intrauterine programming on the development of future diseases. In order to understand the pathogenic mechanisms and natural course of diseases data on body composition is required. Objective techniques of assessing neonatal body composition, such as MRI, DXA and ADP, have been shown to provide accurate and reliable results. The majority of these methods incorporate assumptions (e.g. density of FFM, total lung volume) but as the results are derived after a combination of measurements, the importance of these assumptions is minimized (7, 8). Despite the recent progress in mapping body composition during the early post natal period, objective techniques remain not widely available and are predominantly used for research purposes. To address these limitations, anthropometric equations, based on epidemiological data, have been created and are currently cited in many studies (9, 10).

6.1.1 Anthropometric fat mass equations

There are currently four commonly used equations to estimate FM in infancy (Table 6.1). Variables such as gender, ethnicity, gestational age, length, weight and skinfold thickness are taken into account. Catalano et al (11) used Total Body Electrical Conductivity as the reference method, whereas others (12, 13, 14) have used Air Displacement Plethysmography (ADP). Despite being widely cited, there is limited data on the validation of these equations in specific age groups. As a general rule, equations should be used in age and ethnic groups where they were developed or validated (15).

Table 6.1: Equations for infant fat mass (FM; kgr) estimation. Weight, length and skinfold

Reference	Equation	Reference method	N	Age (days)
Deierlein (12)	$-0.012 - 0.064 * \text{gender} (1 = \text{male}; 0 = \text{female}) + 0.0024 * \text{age} (\text{days}) - 0.150 * \text{body weight (kg)} + 0.055 * \text{body weight}^2 (\text{kg})^2 + 0.046 * \text{ethnicity} (1 = \text{Hispanic}; 0 = \text{not Hispanic}) + 0.020 * \text{sum of 3 skinfolds (triceps, subscapular and thigh)}$	ADP	128	1-3
Catalano (11)	$0.54657 + 0.39055 * \text{Birth weight (g)} + 0.0453 * \text{Flank Skinfold (mm)} - 0.03237 * \text{Length (cm)}$	TOBEC	194	1-3
Lingwood (13)	$\text{FFM} = 0.057 + 0.646 * \text{weight (kg)} - 0.089 * \text{gender} (1 = \text{male}; 2 = \text{female}) + 0.009 * \text{length (cm)}$ $\text{FM} = \text{weight} - \text{FFM}$	ADP	77	0-4
Aris (14)	$-0.022 + 0.307 * \text{weight (kg)} - 0.077 * \text{gender} (1 = \text{male}; 0 = \text{female}) - 0.019 * \text{gestational age (week)} + 0.028 * \text{subscapular skinfold (mm)}$	ADP	88	1-3

thickness from different sites are used to derive FM; Adopted from Cauble et al (15)
ADP: Air Displacement Plethysmography, TOBEC: Total Body Electrical Conductivity

6.1.2 Anthropometric indexes

Anthropometric indexes have been used as practical alternatives and as a proxy to body composition. To be used as a measure of adiposity indexes need to fulfill two criteria: i) they should be correlated with adiposity and ii) they should be independent of association with the denominator (16). Although the first criterion is widely investigated, studies addressing the second are limited (17, 18). BMI ($\text{weight}/\text{length}^2$), ponderal index ($\text{weight}/\text{length}^3$), weight for length, waist circumference for length and the sum (total adiposity) or ratio (central adiposity) of sub scapular and triceps skinfold thickness are the most commonly used body composition indexes (19).

6.1.3 Hypothesis

Defining body composition during the early stages of life would allow a better understanding of intrauterine programming and the association with future metabolic risk. As objective measures of assessing body composition are not yet widely available, fat mass equations using skinfold thicknesses and anthropometric indices are used as surrogate markers of adiposity. We hypothesize that these markers are closely related and in agreement with body composition measurements obtained by a reference method, PEAPOD.

6.1.4 Research questions

- How good are the associations between the commonly used fat mass equations, anthropometric indices and measurements obtained by PEAPOD?

6.1.5 Outcome

- Compare the data derived from the use of fat mass equations and anthropometric indexes are similar to these obtained from PEAPOD

6.1.6 Criteria

The current analysis includes participants from the main longitudinal, observational cohort study described in chapter 3. For this reason inclusion and exclusion criteria remain the same as already described before.

6.1.7 Methods – Statistical analysis

Data from the main longitudinal, observational cohort study was reviewed. Anthropometric measurements obtained at birth and follow up visits were analyzed and combined to calculate anthropometric indexes and FM equations.

Two different FM estimation equations (13, 14) were validated at birth, 4 weeks and 5 months against data obtained from ADP measurements (PEAPOD measurements at birth and during follow up visits). Due to lack of data in our dataset (flank and thigh skinfold thickness) we were unable to validate Deierlein (12) and Catalano (11) equations. All data was assessed for normality. Paired-sample t test was used to detect differences between the estimation equations and the reference criterion. Regression analysis was used to assess the accuracy of each estimation equation against the reference method. To be considered accurate, the regression line should be the same with the line of equality ($x=y$) or in other words the slope of the regression line should not deviate significantly from 1.0. Precision of the equations was assessed using Lohman's criteria (20). R^2 and Standard Error of Estimate (SEE) were calculated for each equation. A SEE between 2 and 3% of fat mass is desirable and classified as very good whereas a SEE >4.0% is considered poor. A R^2 value <0.64 was considered the cut off to define poor agreement between the two methods. Agreement of the methods was also assessed using Bland Altman analyses. Mean difference and 95% limits of agreement were calculated (21).

In order to evaluate which anthropometric index best predicted FM and FFM at birth, 4 weeks and 5 months, multiple linear regression models were created. %FM, FM and FFM values measured by PEAPOD were assessed for normality and constituted the independent variables; anthropometric indexes were the explanatory. BMI, ponderal

index (PI), weight to length (Wt/length), waist circumference to length (WC/length) and sum of triceps and subscapular skinfold thickness (TR+SS) were examined. Models were adjusted for gender, gestational age or postnatal age at estimation. Statistical analysis was performed using SPSS version 22.

6.2 Results

A consort diagram of available data is detailed in Figure 6.1. Infant anthropometry at birth, 4 weeks and 5 months is presented in Table 6.2.

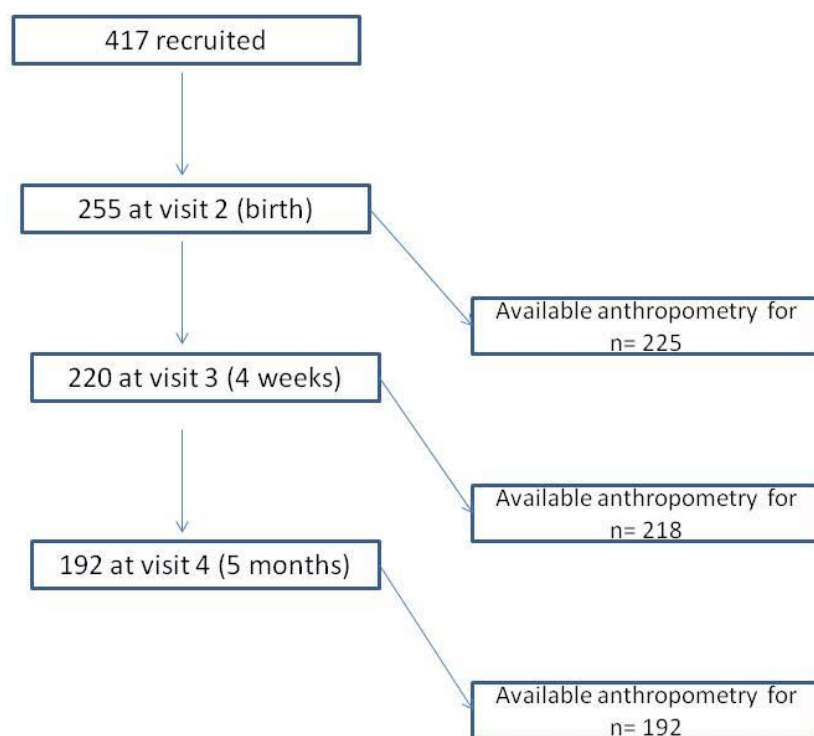


Figure 6.1: Consort diagram of available anthropometry during study visits. Data from 192 neonates were assessed

	Birth (n=225)	Visit 3 (n=218)	Visit 4 (n=192)
Age (days)	2 ± 0.3	32 ± 4	158 ± 20
Gender	M: 102, F: 123	M: 99, F: 119	M: 90, F:102
Ethnicity	Europeans: 191 Asians: 30 Africans: 4	Europeans: 188 Asians: 26 Africans: 4	Europeans: 164 Asians: 24 Africans: 4
Length (cm)	49 ± 2	54 ± 2	65 ± 2
Triceps (mm)	7.0 ± 2.8	8.8 ± 2.1	12.1 ± 3.1
Subscapular (mm)	6.8 ± 2.5	8.6 ± 1.9	10.4 ± 2.9
%FM	10.6 ± 4.2	17.4 ± 4.8	26 ± 5.6
FM (kgr)	0.361 ± 0.175	0.770 ± 0.282	1.958 ± 0.562
FFM (kgr)	2.920 ± 0.388	3.577 ± 0.442	5.496 ± 0.642

Table 6.2: Maternal-Infant characteristics and measurements by reference criterion (PEAPOD) at birth, visit 3 (4 weeks) and visit 4 (5 months)

6.2.1 Fat mass at birth

At birth, significant mean difference was found between the reference criterion and Lingwood equation ($p < 0.01$), whereas Aris equation provided similar results to reference method ($p = 0.1$; Table 6.3). After applying regression to assess accuracy and precision, we found that regression slope differed from 1.0 for both equations. Poor agreement was found between criterion and the two equations with very low R^2 values and high SEE values ranging from 0.175 to 0.172 (Table 6.4).

Agreement was further checked applying Bland-Altman analysis (Table 6.4). The mean difference between criterion and Lingwood equation was -0.4 kgr with 95% limits of agreement of -0.85 kgr, 0.05 kgr. Mean difference between criterion and Aris equation was 0.02 kgr. 95% limits of agreement were -0.488 kgr, 0.448 kgr. There was no proportional bias detected for any of the equations (Figure 6.2).

6.2.2 Fat mass at 4 weeks (visit 3) and 5 months (visit 4)

Mean differences ($p < 0.05$) were found between the criterion and the equations at 4 weeks and 5 months measurements. The slope measured by regression differed by 1.0 for both equations at both time points. R^2 values remained consistently very low with very high ($>4\%$) SEE values. Bland-Altman analysis revealed no agreement between either of the equations examined and reference method (Table 6.4).

Method	FM (kgr)		
	Birth	Assessment 2 (4 weeks)	Assessment 3 (5 months)
ADP (reference)	0.361 ± 0.175	0.770 ± 0.282	1.958 ± 0.562
Lingwood	0.792 ± 0.166	1.130 ± 0.205	2.113 ± 0.357
Aris	$0.334 \pm 0.169^*$	0.699 ± 0.220	1.681 ± 0.355

Table 6.3: Comparison of fat mass (FM) assessment by reference method (PEAPOD) and equations at birth, 4 weeks (visit 3) and 5 months (visit 4). Aris equation provided similar FM values with PEAPOD at birth but not at follow up visits

* p value > 0.05 (non significant difference from the criterion method)

Reference criterion vs Equation	Regression analysis			Bland-Altman	
	Slope	R^2	SEE	Mean dif.	95% limits of agreement
Birth					
Lingwood	0.02	0.001	0.175	-0.40	-0.85, 0.05
Aris	0.01	0.001	0.172	0.02	-0.44, 0.48
4 weeks					
Lingwood	0.09	0.005	0.282	-0.35	-0.99, 0.29
Aris	0.1	0.008	0.283	0.68	0.02, 1.34
5 months					
Lingwood	0.07	0.002	0.497	-0.15	-1.42, 1.12

Table 6.4: Comparison of criterion method (PEAPOD) and fat mass (FM) equations using regression and Bland Altman analysis. Data revealed persistently low R^2 and high SEE values, suggesting poor agreement between equation and reference method

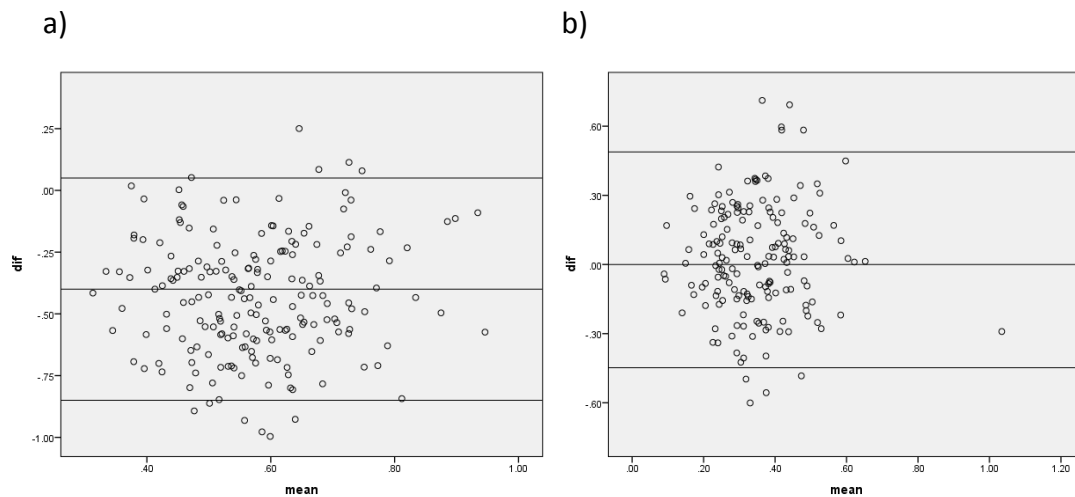


Figure 6.2: Bland Altman plot of fat mass (FM) predicted by a) Lingwood and b) Aris equations against reference criterion (PEAPOD) at birth. Middle line represents the mean difference between equation studied and reference criterion and upper/lower lines represent 95% limits of agreement

6.2.3 Prediction of body composition by anthropometric indexes

We used multiple regression models adjusted for gender, gestational age and age at assessment. Five different indexes were compared at birth, 4 weeks and 5 months post partum. FM, %FM and FFM obtained from PEAPOD measurements were used as reference values.

Table 6.5 shows the relationship between anthropometric indexes and body composition. At birth, PI was the best predictor of FM ($R^2=0.07$) and %FM ($R^2=0.06$) whereas WC/length best predicted FFM ($R^2=0.21$). At 4 weeks, WC/length remained the best predictor of FFM ($R^2=0.03$) but was also superior in predicting FM ($R^2=0.02$) and %FM ($R^2=0.03$). At 5 months, the analysis revealed SS+TR as the best predictor of FM ($R^2=0.03$) and %FM ($R^2=0.03$).

Data in Table 6.5 reveal that the relationship between anthropometric indexes and body composition was very weak at all time points, with values ranging from 0.006 to 0.22. Consistent with the above observation, the effect size on body composition remained extremely low for all indexes, as indicated by the β co-efficient value. All indexes consistently explained a higher variation in FFM values compared to FM and %FM.

a)

Index	FM		%FM		FFM	
Birth	β coef.	R ²	B coef.	R ²	B coef.	R ²
BMI (kg/m ²)	0.01	0.05	0.28	0.04	0.01	0.19
PI (kg/m ³)	0.005	0.07	0.14	0.06	0.006	0.19
Wt/length (kg/m)	0.01	0.05	0.38	0.04	0.03	0.19
SS+TR (mm)	0.003	0.05	0.05	0.04	0.005	0.20
WC/length (cm/m)	0.003	0.04	0.06	0.03	0.01	0.21

b)

Index	FM		%FM		FFM	
4 weeks (visit 3)	β coef.	R ²	B coef.	R ²	B coef.	R ²
BMI (kg/m ²)	0.02	0.01	0.49	0.02	0.019	0.03
PI (kg/m ³)	0.01	0.01	0.22	0.02	0.008	0.033
Wt/length (kg/m)	0.01	0.006	0.27	0.009	0.002	0.02
SS+TR (mm)	0.003	0.004	0.05	0.001	0.01	0.005
WC/length (cm/m)	0.01	0.02	0.19	0.03	0.003	0.035

c)

Index	FM		%FM		FFM	
5 months (visit 4)	β coef.	R ²	B coef.	R ²	B coef.	R ²
BMI (kg/m ²)	0.001	0.004	0.19	0.03	0.05	0.148
PI (kg/m ³)	0.001	0.005	0.05	0.03	0.01	0.130
Wt/length (kg/m)	0.01	0.006	0.28	0.03	0.03	0.125
SS+TR (mm)	0.01	0.03	0.14	0.05	0.007	0.125
WC/length (cm/m)	0.003	0.007	0.003	0.03	0.02	0.141

Table 6.5: Relationship between body composition measures obtained with PEAPOD and anthropometric indexes at a) birth, b) 4 weeks (visit 3) and c) 5 months (visit 4). All indexes presented low R² values with weak effect size on FM and %FM at three different time points.

6.3 Discussion

In this chapter we assessed the validity of FM equations against a reference method, PEAPOD. We also sought to investigate which anthropometric index best predicts body composition components up to 5 months of age. Our results show: i) Poor agreement between Lingwood (13) and Aris (14) FM equations and the reference criterion ii) PI is a better predictor of FM at birth but WC/length ratio has a stronger correlation with FM at 4 weeks iii) All anthropometric indexes examined have a very low effect size on body composition and consistently explain a higher variation in FFM compared to FM.

6.3.1 Validity of FM equations

In this analysis we assessed the validity of Lingwood et al (13) and Aris et al (14) equations in predicting FM against PEAPOD measurements, which was used as the reference method. At birth, there was no mean difference between Aris equation and the criterion. At follow up assessments, mean differences were detected for both equations and the reference method. Both equations had low accuracy and precision for all time points as indicated by regression analysis. Results revealed that slope was different from 1.0 at all assessments and the R^2 and SEE values were consistently low and high respectively. Bland-Altman analysis revealed poor agreement between the equations and the criterion with wide 95% limits of agreement.

As objective measurements of body composition are not yet widely available, prediction equations have been created to provide useful tools during clinical assessment. Equations presented in Table 6.1 have been recently developed and are increasingly cited when assessing infant FM. Deierlein et al (12) equation incorporates data from thigh skinfold thickness whereas Catalano et al (11) uses data from flank skinfold thickness. In our cohort skinfold thickness was assessed only at two sites, sub scapular and triceps, therefore due to lack of data in our dataset we were unable to assess those two equations. To our knowledge, Lingwood and Aris equations have only been validated once, using PEAPOD measurements as the reference method. Similar to our results, Cauble et al (15) showed poor agreement between the equations and the criterion.

In an attempt to explain our results, we explored the main principles of the equations. Anthropometric equations are created based on large epidemiological studies. One of the main limitations is that equations should be applied only to groups which share similar characteristics (age group, ethnicity) to the reference population. Both equations examined in this chapter were based on neonatal data (1-3 days old) and were not

validated in older infants. This could explain the poor agreement found at 4 weeks and 5 months follow up assessments but not the discrepancy at birth. Lingwood equation was based on a study on Australian population, of which 90% were of white European descent, whereas Aris equation was developed from Asian population living in Singapore, including Chinese, Indians and Malay neonates. Neither of these equations takes ethnicity into account. Our cohort comprises neonates from a multi ethnic background with 85% being White British and 15% Asians and Africans. Ethnic diversity in body composition is well described (22), therefore the mismatch between the studied and validation population could have potentially biased the results.

Finally, FM equations are based on anthropometric measurements, such as height, weight and skinfold thickness. Measurements are prone to intra- and inter-observer variability and infancy is a challenging period to acquire precise readings. Small deviations from the actual infant size would cause great diversity in the estimated FM. A detailed comparison between our study and data presented from the Kansas cohort (15) proves our hypothesis (Table 6.6). Neonates in the Kansas group were found to have lower triceps (5.6 ± 1.5 vs. 7.0 ± 2.8 mm) and sub scapular (5.3 ± 1.4 vs. 6.8 ± 2.5 mm) skinfold thickness compared to our study but the absolute (0.374 ± 0.170 vs. 0.361 ± 0.175 kgr) and %FM ($11.2 \pm 4.3\%$ vs. $10.6 \pm 4.2\%$) were found to be disproportionally higher when measured by PEAPOD. Assuming that PEAPOD measurements were performed under adiabatic conditions and as per operating protocols at both sites, differences can be explained by experimenter error or bias in obtaining the skinfold results. In our study, skinfold measurements were performed by the author, two research midwives and a research assistant. All staff was trained to obtain anthropometric measurements as indicated by WHO Multicentre Growth Reference Study (MGRS) and INTERGROWTH-21st anthropometry handbook (23).

	PEAPOD study	Kansas Group
Maternal age (yrs)	32.1 (5.4)	28.9 (4.8)
Maternal BMI (kgr/m²)	30.3 (6.7)	25.8 (6.1)
Gestational age (weeks)	39.0 (2.6)	39.2 (2.8)
Birthweight (kgr)	3281 (0.499)	3497 (0.404)
Triceps skinfold (mm)	7.0 (2.8)	5.6 (1.5)
Subscapular skinfold (mm)	6.8 (2.5)	5.3 (1.4)
%FM	10.6 (4.2)	11.2 (4.3)
FM (kgr)	0.361 (0.175)	0.374 (0.170)

Table 6.6: Comparison of mean values (SD) between our cohort and data obtained by the Kansas group (15). The discordant results between skinfold thickness and objectively measured adiposity (PEAPOD) suggest intra and inter observer variability in obtaining anthropometric values

6.3.2 Anthropometric indexes as predictors of body composition

We have presented data showing that PI and WC/length are the best predictors of FM and FFM at birth respectively. We were unable to demonstrate consistency of the results found at birth across other age groups. Specifically, we found that WC/length ratio best predicted all body composition components during the 4 weeks assessment but SS+TR was a better predictor of FM at 5 months. We have also shown that anthropometric indexes explain only a small proportion of the variation in body composition as indicated by the low R^2 values, therefore their use in clinical practice remains questionable. Furthermore we have shown that these indexes are more closely correlated to FFM rather than FM across all age groups.

In 1970 Keys et al (24) was the first to evaluate weight-height indexes as measures of adiposity. Ten years later the International Dietary Energy Consultancy Group (IDECG) task force introduced BMI as a marker of “energy deficiency” (25). Since then, there has been a lot of controversy regarding the ideal index to describe adiposity. An anthropometric index should be able to identify any positive or negative correlation with adiposity but at the same time needs to be statistically robust to allow risk stratification. In other words, the ideal index should be closely related to adiposity and be independent of association with denominator (16). BMI and PI use the power of height in an attempt to eliminate any correlation with the denominator. BMI is highly correlated with weight but increasing evidence suggests that it is a poor index of fat, especially in children (26). Consistent with our findings, changes in weight/length^p values are more likely to reflect

FFM rather than FM changes, an observation more likely explained by the rapid developmental growth up to adolescence (5). Furthermore, both indexes cannot be used interchangeably across all age and ethnic groups. The optimal exponent for weight/length^p ratio changes from 3 (PI) at birth to 2 (BMI) in adulthood (27).

Evidence from cross-sectional studies confirms that the optimal anthropometric index is yet to be identified. Data from the INTERGROWTH 21st study suggests that the weight/length ratio is the best predictor of FM and FFM at birth in one of the largest, multi-ethnic cohorts studied ever (19). On the other hand, Cole et al. suggested that BMI is superior to simple weight/length ratio after studying 40,500 Dutch children (28). The same group described the weight/length ratio as statistically inappropriate due to high correlation with the denominator (29). Finally, reports from two smaller cohorts suggest the use of WC/length ratio (18) and the skinfold thickness (17) as optimal markers of adiposity.

In our cohort we were unable to find a FM equation agreeing with our reference criterion and we failed to demonstrate an index which can strongly predict body composition throughout infancy. We strongly believe that our findings, as well as the controversial data derived from the studies described before, are due to the dynamic changes occurring in the early post natal period. Infancy is a period of rapid growth with great inter-individual variability. Infants are expected to increase their length by 30% by 5 months and by 50% by 12 months. At the same time, they are expected to lose up to 10% of their weight during the first 5 days and regain it by the end of the second week. Birthweight typically doubles by 5 months and triples by 12 months (30). Gender, ethnicity and feeding mode could be potential contributors to this variability. Females are known to have more FM and less FFM for a given weight when compared to males (31). In our previous chapters we have demonstrated that this gender dimorphism in body composition is present from the early stages of life. At the same time, specific populations, such as South Asians, are known to have higher FM for a given BMI (32), implying that universal application of FM equations and anthropometric indexes is unlikely to provide reliable findings. Feeding mode has not been addressed in any of the equations examined. Although data on body composition and feeding mode remain controversial, it is widely accepted that formula fed infants are more likely to lose less weight during the first 5 days of life (33). Finally, to further support our theory evidence shows a better agreement between equations and actual body size in childhood, adolescence and adulthood, periods where growth and body composition are more stable than infancy (34).

6.4 Conclusion

Currently used FM equations lack validation studies and are inappropriately used to estimate body composition. Our findings suggest that these equations lack accuracy and precision, leading to unreliable results during clinical assessment. The “gold standard” anthropometric index is yet to be identified. Commonly used indexes are not consistently correlated with adiposity across various study groups and some of them may be statistically flawed. Both FM equations and anthropometric indexes rely on measurements which are prone to intra- and inter-observer variability. Tracking the origins of adult disease from the early stages of life requires better understanding of body composition and objective measurements of adiposity, such as ADP and DXA, could be potential alternatives in the future.

6.5 References

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Chapter 7

Summary and Conclusions

7.1 The origins of childhood obesity

Childhood obesity is one of the major public health concerns of our century and is related to immediate (1, 2) and long term cardiovascular, metabolic and psychological adverse outcomes (3). Latest data from the European Childhood Obesity Surveillance Initiative (COSI) reveals that 25-30% of 13.7 million children aged 6-9 years are either overweight or obese (4). Severe obesity affects 400,000 children of school age in 21 European countries (Figure 7.1). Results are similar in England where 20% of children are affected by obesity by the age of 6 years (5). Secondary analysis of the data shows that parental obesity, low educational and socioeconomic status are linked to higher rates of childhood obesity. On the other hand, countries experiencing nutritional transition have lower rates. Longitudinal evidence support that childhood obesity is related to adverse phenotype and metabolism in adulthood (6). Data from 2017-2018 in England reveals that 10,660 hospital admissions were attributed to obesity causing substantial costs to the NHS, with 16 billion a year spent to conditions related to obesity (5). Currently in the UK, the number of adults with obesity is two times higher than those who are smoking. Given that childhood obesity has a huge impact on health, well being and economy it is of vital importance to tackle the epidemic by various approaches, from prevention to early diagnosis and treatment.

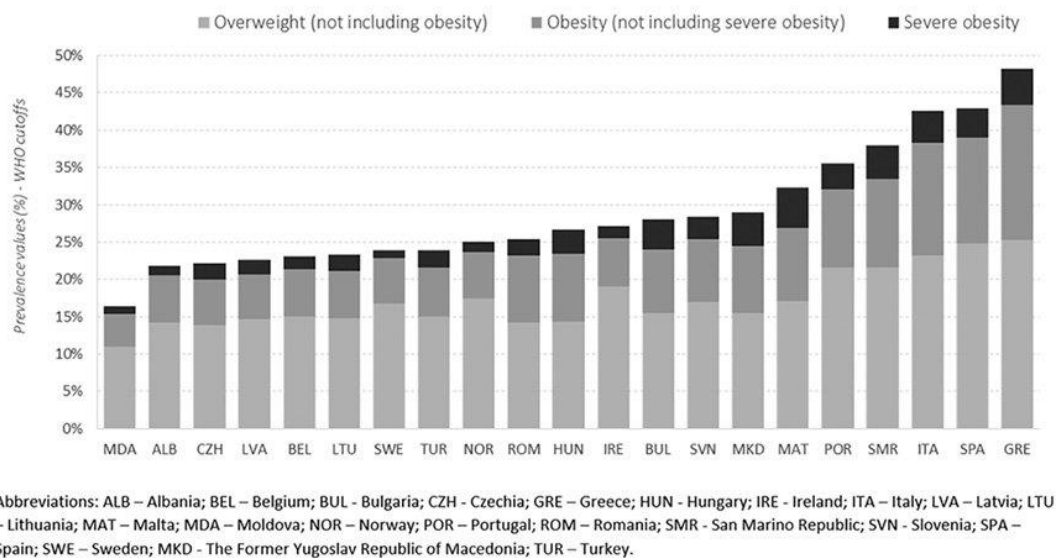


Figure 7.1: Prevalence of childhood overweight, obesity and severe obesity in Europe; Adopted from Spinelli et al (4)

The development of obesity is complex and multifactorial (Figure 7.2). It is a result of energy imbalance that occurs when caloric intake exceeds expenditure. Characteristics

of modern societies such as unhealthy diet, low levels of physical activity and sedentary lifestyle have contributed to the obesity outburst. However, neither all people living in these environments will become obese nor all obese people will develop same adverse outcomes. The above observations suggest a role of genetic predisposition, with adverse phenotype developing when subjects are exposed to “obesogenic challenges”. Indeed, up to 40% of offspring BMI may be heritable (7) and epidemiological data suggests that in 25% of cases of childhood obesity at least one of the two parents is also obese or overweight (5). As genetic pool remains unchanged (or changes with very slow rate) the current obesity epidemic cannot be entirely attributed to genetic factors. Compared with siblings born before mother underwent bariatric surgery, siblings born after maternal weight loss display lower rates of obesity and better metabolic profile (8). The above highlight the role of intrauterine programming. Events occurring in the “plastic developmental period” metabolically imprint offspring for the rest of life.

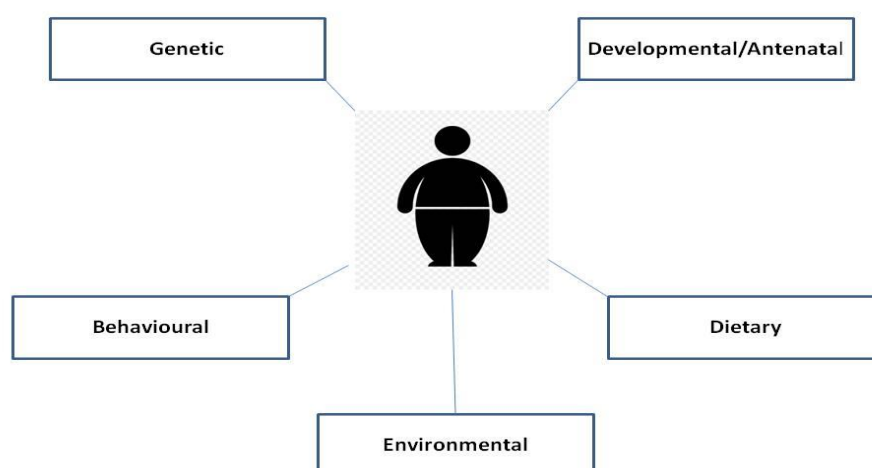


Figure 7.2: Determinants of childhood obesity. Genes, antenatal and postnatal exposures contribute to the pathogenesis of adverse phenotype

Fetal growth is dependent on maternal environment and changes in nutritional, hormonal and metabolic conditions can affect the development of organs and gene expression. Barker et al (9) was the first to focus on the effects of maternal undernutrition and chronic cardiovascular disease. The “thrifty phenotype” hypothesis was further supported by epidemiological data from the Dutch famine (10). More recently, maternal overnutrition has been added to the developmental Origins of Health and Disease (DOHaD) concept (11). Maternal characteristics consistent with the lifestyle of modern days, such as obesity, increased GWG and impaired glucose metabolism, seem to be associated with

future obesity and metabolic disorders. The early life programming of future diseases is a strong indicator that prevention strategies should be implemented in the pre-conception and antenatal period. The success of a pioneering prevention program aiming to improve offspring health via improving maternal health depends on the understanding of the pathophysiological mechanisms underpinning the developmental origins of metabolic diseases. Therefore it is of great importance to answer the following questions:

- What is the missing link between adverse maternal characteristics and offspring obesity?
- What are the mechanisms involved in the process of intrauterine programming?
- How can we identify high risk groups from the early stages of life?
- When is the best period to prevent or reverse adverse intrauterine programming?

Purpose of the current thesis was to shed light to these questions and provide insight for further research.

7.2 Maternal BMI, gestational weight gain (GWG) and glycaemia

The parallel increase of maternal and childhood obesity was the first clue that maternal BMI may be a developmental factor (12). Higher maternal BMI translates to higher birthweight (BW), which in turn is strongly related to childhood obesity (13). Of great interest though is the fact that maternal BMI has a greater predictive value on future obesity than BW (14). The above observation confirms that BW is a rough estimate of fetal growth and detailed neonatal body composition is required to better understand the programming effect of maternal obesity. Up to date, the majority of studies assess the effect of maternal obesity using BW or skinfold derived FM equations as the outcome variable. In our study we assessed infant body composition using a direct measure, PEAPOD. We demonstrated that maternal BMI predicts higher neonatal adiposity up to 5 months of age, independent of other maternal factors (GWG, glycaemic status).

The optimal GWG has been an area of controversy for many years. In 2009, IOM published guidelines on optimal weight gain to support adequate fetal growth and optimize maternal and neonatal outcomes (15). The role of GWG on adverse metabolic programming has received a lot of attention in the last decade but data on the effect of neonatal body composition remains scarce. Combined data from Europe, North America and Australia suggests that GWG above IOL recommendations explains up to 20% of childhood obesity (16). Results were consistent with subgroup analysis of the HAPO participants in China which demonstrated that excessive GWG is related to childhood obesity, hypertension and increased insulin resistance (17). The same group was found

to have increased adiposity and cord c-peptide levels at birth. Our study provides strong evidence that independent of maternal BMI, GWG is related to FM accumulation during the first 5 months of age. The vast majority of our participants were either overweight or obese therefore we were unable to assess the effect size of GWG based on maternal BMI. Previous studies consistently have shown that the effect of GWG on offspring outcomes attenuates as maternal BMI increases (16). One of the striking findings of our study is that GWG below IOM recommendations is related to lower FFM at birth. Similarly, Catalano et al, using neonatal anthropometry, showed that GWG < 5kg is related to lower neonatal FFM (18). The fact that FFM is the most metabolically active tissue in human body (19) paired with the theory of collateral fattening in cases of FFM deficit (20) (theory will be analyzed later in this session) could potentially explain the increased adiposity and insulin resistance at 6 years of age, showed by the HAPO subgroup analysis from Hong Kong (Figure 7.3).

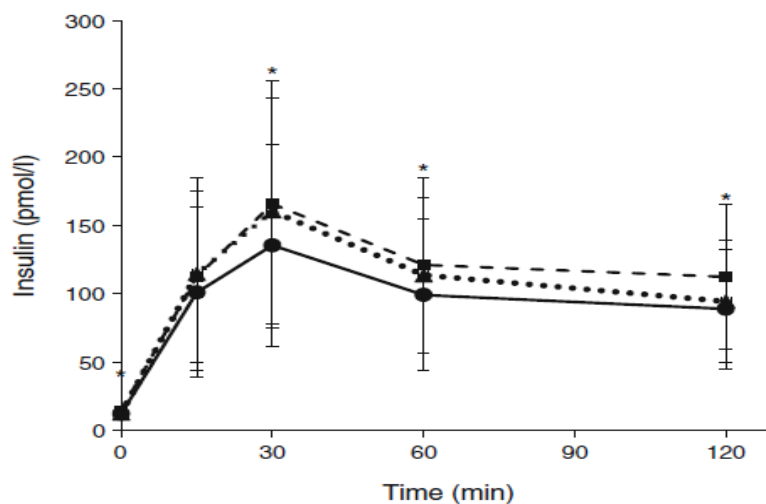


Figure 7.3: Insulin concentrations at different time points during OGTT at 6 years old children, stratified by maternal BMI according to IOM recommendations. Gestational weight gain below recommendations has been proven to be equally harmful to excessive weight gain; Black circles: within recommendations; Triangles: below recommendations; Squares: exceeding recommendations; Adopted from Tam et al (17)

The effect of maternal hyperglycaemia on fetal growth has been widely reviewed with the most robust data presented from the HAPO study (21). Maternal BMI is closely linked with glucose metabolism with maternal obesity related to higher fasting and post-prandial glucose levels (22). The above observation has raised the suspicion that increased adiposity at birth is not related to glycaemic status per se but more likely reflects the effect of maternal obesity. Our data confirms the independent effect of fasting and post prandial hyperglycaemia on neonatal FM, after adjusting for maternal characteristics including BMI. Furthermore, we provide evidence that the association persists at sub-

diabetic glucose levels, showing a continuous relationship between maternal glucose and adiposity at birth. In our cohort, fasting glucose levels (OGTT₀) seem to have a stronger effect on FM at birth when compared to post glucose load (OGTT₁₂₀).

7.3 What is the underlying pathogenic mechanism?

In an attempt to identify the missing link between maternal adverse characteristics and neonatal adiposity we examined maternal and cord blood biomarkers. We demonstrated that maternal BMI is associated with higher glucose levels, higher insulin resistance and higher leptin levels. We also observed a trend between maternal adipocytokines and neonatal adiposity, which is positive in the case of leptin and negative in the case of adiponectin. We have shown that maternal BMI, glucose levels during OGTT and insulin resistance independently predict neonatal body composition.

After examining cord blood biomarkers we found that cord adipocytokines and c-peptide are related to each other and with neonatal adiposity. Given that insulin is the main growth factor during fetal development (23) and maternal insulin cannot cross the placenta (24) we looked at associations between maternal factors and fetal insulin status. Fetal hyperinsulinaemia was correlated with maternal insulin resistance but not with maternal BMI or glucose levels during OGTT. Taking into consideration the above observations we support that insulin resistance is the major metabolic factor underpinning the relationship between maternal intrauterine environment and body composition at birth.

Pregnancy is a period of “hormonal challenges” for the human body. Placental hormones, such as estradiol, progesterone and placental lactogen promote insulin resistance in order to secure adequate nutritional supply to the growing fetus (25). In cases of maternal obesity the insulin resistance is exaggerated due to the maternal pre-pregnancy metabolic status. Similarly, excessive GWG is related to higher insulin resistance. Although weight gain in pregnancy occurs to support the developing fetus, excessive weight gain results in central fat accumulation which promotes insulin resistance (26). As a result of reduced insulin sensitivity, lipogenesis and protein synthesis are suppressed and hepatic gluconeogenesis is enhanced. Circulating levels of glucose, amino acids and free fatty acids cross the placenta and stimulate fetal insulin secretion. Given the anabolic effect of insulin and the preferential action on FM rather than FFM (27), fetal hyperinsulinaemia leads to adiposity and macrosomia, an effect known as “fuel mediated teratogenesis” (Figure 7.4). The effect of insulin resistance,

independent of maternal BMI and glucose tolerance, on neonatal adiposity has been previously shown by other researchers (28-30). Of great importance, transmission of obesity to offspring post bariatric surgery is reduced even if mothers remain overweight, suggesting that insulin resistance, rather than maternal BMI, is the responsible pathogenetic factor (31).

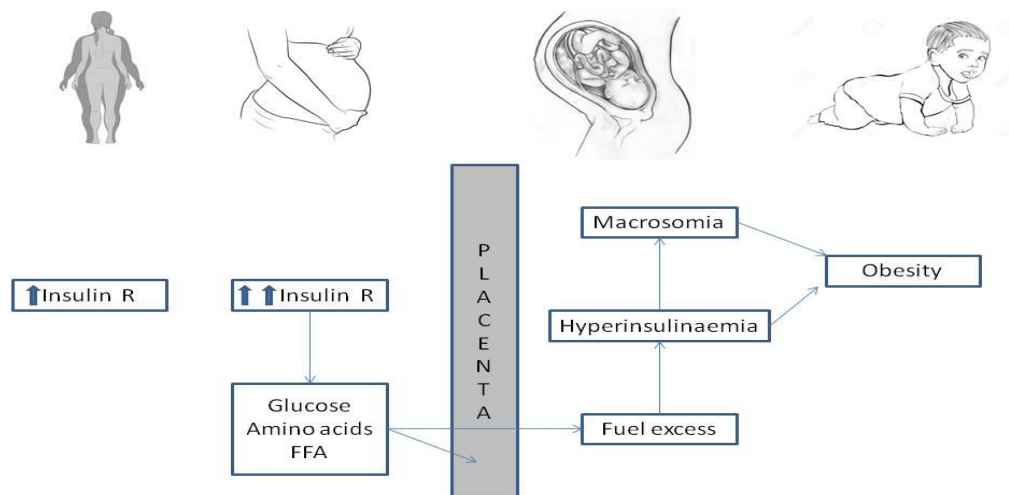


Figure 7.4: Fuel mediated teratogenesis. Glucose, proteins and lipids transplacental transport affects offspring adiposity

7.4 What about maternal characteristics and childhood obesity?

The correlation between maternal BMI, GWG and future risk of obesity is complex. Maternal metabolic status can affect offspring metabolic trajectories via genetic factors, intrauterine programming, epigenetic effects and shared post natal obesogenic environment. Furthermore, it is difficult to quantify the effect of GWG as it reflects not only maternal nutritional affluence but also the growth of fetus, placenta and uterus. In addition, a great proportion of GWG is attributed to fluid retention. On the other hand, the correlation between maternal adverse characteristics and future obesity, traced from infancy to adulthood suggests a role of developmental programming.

We demonstrated that cord leptin and c-peptide levels reflect fetal FM and are also related to maternal insulin resistance. We suggest that fetal/neonatal hyperleptinaemia and hyperinsulinaemia are important programming factors of future obesity. In humans, hypothalamus, the main centre for satiety and energy expenditure control, develops during the antenatal and early post natal life. Leptin and insulin display neurotrophic

actions and are required for the correct formation of neuronal projections (32). Evidence from animal models suggests that prolonged exposure to supraphysiological levels of leptin (33) or insulin (34) alter hypothalamic structure and function, promoting the development of orexigenic over anorexigenic neurons in the ARC. As a result, offspring develop hyperphagia, obesity and impaired glucose metabolism. At the same time, data reveals that the early post natal period is characterized by “plasticity and reversibility” and exposure to favorable environment could potentially revert any adverse metabolic programming (35). We speculate that maternal insulin resistance adversely programs the hypothalamus via increased fetal hyperleptinaemia and hyperinsulinaemia leading to future metabolic dysfunction.

7.5 Answering the hard questions

Interventions during pregnancy (diet, physical activity), despite reduction in GWG, have little effect on pregnancy outcomes (GDM, HTN) and fetal overgrowth (birthweight, LGA). It has been suggested that wide variability of the interventions and their intensity, poor compliance of the case group and cross-motivational effect on the control group may explain the results. At the same time, interventions starting at the pre-conception period have shown promising results (36, 37). In animal models diet interventions before pregnancy normalize neonatal adiposity, leptin and insulin levels leading to improved metabolic profile in adulthood (38, 39). The above observations lead to the following questions: i) Do we intervene too late? ii) Does maternal adverse metabolism affect developmental programming during early pregnancy?

The intrauterine development of adipose tissue is summarized in Figure 7.5. Adipose tissue is not present before 14 weeks of gestation. At that point aggregation of mesenchymal cells starts from the head to the trunk and finally to the limbs. Around 16 weeks of gestation the first pre-adipocytes are formed. The period between 19-28 weeks of gestation is characterized by maturation of adipocytes, followed by a period of rapid adipose tissue accumulation until term (40). Of importance, between 23 and 28 weeks of gestation the number of adipocytes remains constant and it is only the size which changes. Therefore, the window between 14-23 weeks of gestation may be characterized by developmental plasticity with adverse events affecting proliferation and differentiation of adipocytes (41, 42). In fact, evidence from animal models suggest that nutritional and hormonal signals in early pregnancy upregulate proliferation and PPAR γ expression (differentiation) leading to adipose tissue accretion (43, 44).

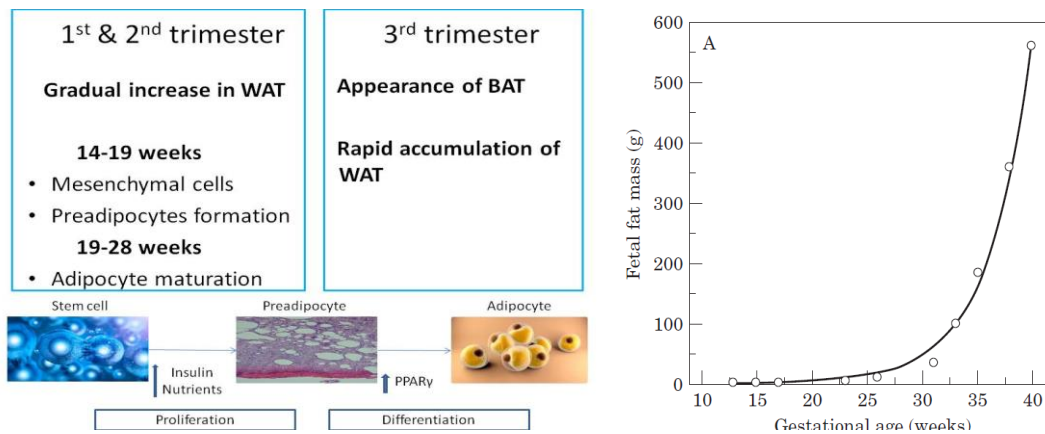


Figure 7.5: Fetal adipose tissue development. Adipocytes present at 14 weeks of gestation and accumulate rapidly during the third trimester

In the last decade, accumulating evidence suggests that maternal metabolic status during preconception and early pregnancy plays a crucial role in offspring metabolic imprinting through altered placental growth and epigenetic modifications (45). Placenta is a complex organ which acts as a nutrient sensor in an attempt to match maternal supply with fetal requirements. Placental weight is strongly related to neonatal adiposity (46) and mothers with obesity are known to have heavier placentas (47). Once again, the link underpinning the above association is increased maternal insulin resistance (Figure 7.6). Maternal hyperinsulinaemia affects mitochondrial function (48) and leads to hyperplastic placenta (49). In turn, the excess of placental hormones increases maternal insulin resistance further, leading to unopposed transfer of nutrients to fetal circulation and macrosomia. Furthermore, hyperinsulinaemia is related to placental over-expression of amino acid and lipid transporters contributing further to the excessive fetal supply (50).

Human's phenotype is determined not only by the DNA sequence but also by information inherited due to gene expression, the later mechanism known as "epigenome". During gametogenesis genes are expressed or silenced leading to the inheritance of a new regulatory state. DNA methylation is the most described epigenetic mechanism (51). Maternal obesity is linked to altered methylation of the PPAR γ promoter in fetuses resulting in increased adipogenesis (52, 53). Children born to mothers before and after bariatric surgery present differences in the methylation of the glucoregulatory gene. (54) Furthermore, maternal hyperglycaemia is related to altered DNA methylation in the fetal liver and pancreas, causing adverse epigenetic programming (55, 56). Tracing epigenetic modifications in offspring with obesity from birth to early adulthood has revealed that methylation sites at birth may attenuate, disappear or be replaced by new sites (57). The above observation proves that DNA methylation occurs during the whole life spectrum

and can also be mediated by environmental factors. Body composition and metabolic changes at birth, such as neonatal insulin resistance and adiposity may drive physiological pathways causing obesity later in life, even if the related epigenetic mechanisms attenuate. At the same time, early post natal life is the right period to intervene to reverse any adverse metabolic programming.

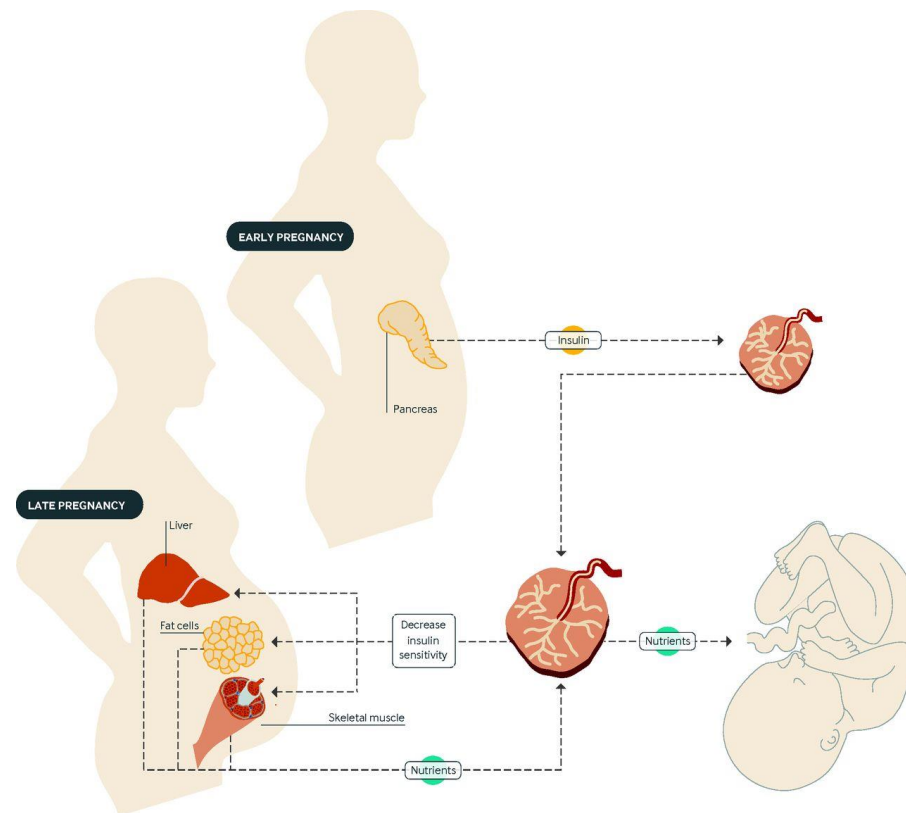


Figure 7.6: Preconception maternal hyperinsulinaemia and fetal overgrowth. Hyperinsulinaemia can affect placental growth but also transplacental nutrient transport resulting in fetal macrosomia and adiposity; Adopted from Catalano et al (45)

7.6 Putting everything together

Maternal obesity is characterized by metabolic derangements and through genetic and lifestyle differences is linked to increased adiposity, gestational weight gain, hyperglycaemia and insulin resistance. The developmental programming occurs throughout pregnancy and early postnatal life and lifelong metabolic imprinting is mediated through altered tissue structure and function (Figure 7.7). Exposure to maternal obesity and increased insulin resistance in early gestation causes altered placental growth and epigenetic alterations to key organs (liver, muscle, adipose tissue) setting adverse metabolic trajectories for the offspring. Throughout pregnancy, the increased maternal insulin resistance results in excessive fetal nutritional supply leading to neonatal

macrosomia and adiposity. The increased fetal insulin and leptin levels, caused either by nutritional stimulation and fat mass accretion or by overexpression of genes (epigenetic mechanism), alter hypothalamic structure leading to hyperphagia, obesity and impaired metabolism in the future.

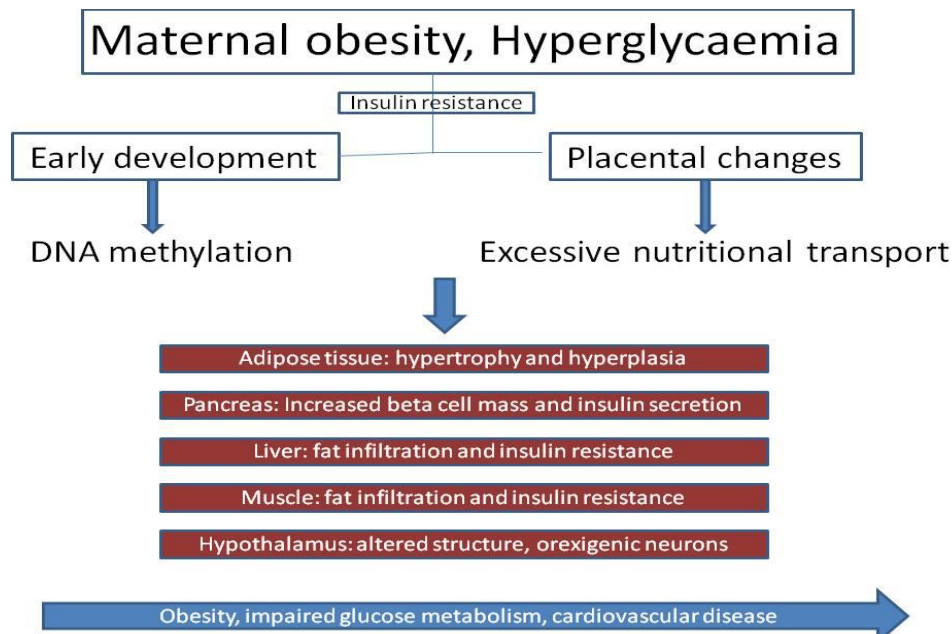


Figure 7.7: Structural and molecular changes as a result of adverse intrauterine environment

7.7 Early postnatal life - Is it too late to intervene?

In humans, hypothalamic circuits develop during late pregnancy and early postnatal life. Arcuate nucleus is the regulatory centre of appetite and energy. Metabolic hormones, such as leptin and insulin are crucial for the formation of the hypothalamic circuitry (32). Animal studies suggest that early postpartum is a period of plasticity where interventions can determine future metabolic trajectories (58). Of great importance evidence suggest that “favorable” conditions during early life can alter adverse intrauterine programming leading to better metabolic health in future (35). Knowledge obtained from animal studies provides insight into ways to prevent childhood obesity. Early identification of high risk groups with implementation of prevention strategies from the early stages of life could be the way to tackle the epidemic.

Birthweight and BMI have been traditionally used as markers of obesity. Although easy to obtain, both markers don't precisely reflect intrauterine growth and body composition of the offspring (59). Anthropometric measurements and FM equations using various

skinfold thicknesses have been extensively used by researchers to estimate body composition. However, these measurements are prone to inter and intra observer variability and have not been validated in all age groups (e.g. neonates) (60). Using PEAPOD as the reference method to assess infant body composition we revealed no agreement between the reference method, skinfold thickness and FM equations. Direct techniques of assessing adiposity, such as PEAPOD, MRI and DXA are not widely available due to cost of the equipments (all) and time required (MRI and DXA) for each test and their use is currently restricted to research purposes.

The above observations led us to investigate the role of cord blood biomarkers in predicting future adiposity. Our results show that cord blood leptin, adiponectin and c-peptide are useful objective markers of neonatal FM. Using data from our cohort and evidence from our systematic review we showed that cord leptin is negatively related to adiposity up to 3 years of age with the association becoming positive thereafter. Of great interest, we found that cord blood c-peptide seems to follow the same trend with leptin, at least up to 5 months of age. The negative correlation during the first 3 years of life can be explained by the metabolic profile of leptin. Infants with increased adiposity, thus higher leptin levels, develop a “compensatory behavior” driven by the leptin’s effect on appetite and energy expenditure. The “honeymoon” period of negative energy balance is followed by hyperphagia and fat mass accretion leading to increased weight gain and adiposity by 7 years of age. In animal models, there is evidence that the obesogenic offspring behavior is a result of adverse hypothalamic programming and leptin resistance. Abnormal levels or abnormal surge of leptin and c-peptide during early postnatal life lead to altered hypothalamic structure favoring the development of orexigenic over anorexigenic neurons (33, 34). At the same time, intrauterine exposure to adverse maternal environment leads to altered organ development resulting into increased leptin resistance in liver, muscle and adipose tissue (61). This adverse programming becomes clinically evident in early childhood (Figure 7.8).

The concept of using cord blood biomarkers to identify neonates at high risk of obesity is promising. Samples are obtained directly from the cord, not from the neonate, without requiring any special training for staff. The potential development of “point of care” kits to measure cord blood levels will allow the identification of high risk groups within minutes after birth. Extending our findings to a larger cohort with follow up assessment of body composition in early childhood will provide robust data for the predictive value of cord biomarkers. This will allow targeted implementation of prevention strategies aiming to revert adverse intrauterine programming by early life interventions.

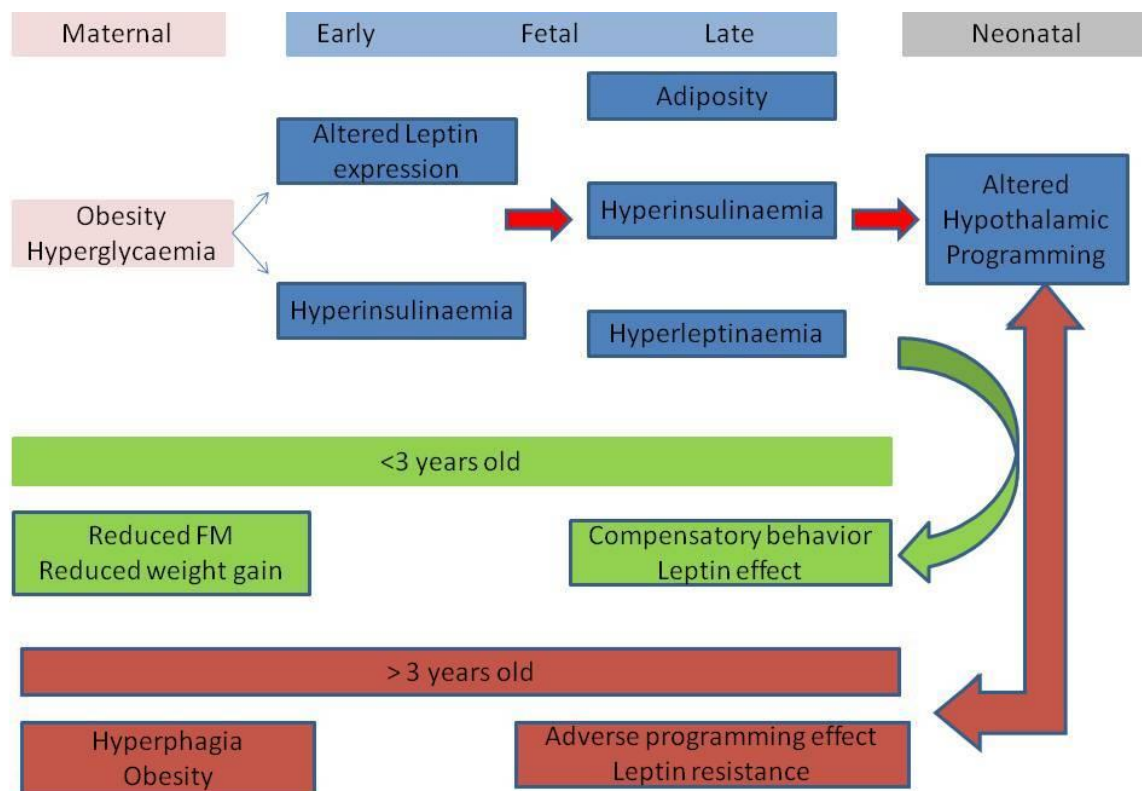


Figure 7.8: Leptin as a predictor of future obesity. Abnormal leptin levels affect hypothalamic development, resulting in early leptin resistance, hyperphagia and adiposity. Neonatal hyperleptinaemia leads to a compensatory behavior during the first 3 years of life

7.8 Gestational diabetes mellitus (GDM)

GDM is defined as glucose intolerance of different degrees that is first diagnosed during pregnancy. The pathogenesis of GDM is a combination of increased insulin resistance and insufficient insulin secretion. In other words, beta cell function is not able to overcome the level of insulin resistance in order to maintain euglycaemia. GDM parallels the obesity epidemic, complicating 2-6% and 9% of pregnancies in Europe (62) and US (63) respectively. There is no universal consensus on optimal screening and diagnostic criteria but it is widely accepted that pregnancies complicated by GDM are related to short and long term adverse outcomes for both mother and offspring. Women who develop diabetes during gestation may already have an impaired metabolic profile which is unmasked due to the stressogenic effect of pregnancy on metabolism. They comprise a high risk population having two times higher risk for cardiovascular disease (64) and at least seven times higher risk for type 2 diabetes mellitus (65). Uncontrolled maternal hyperglycaemia leads to fetal macrosomia, adiposity and increased perinatal adverse

outcomes (21). Strong evidence from the HAPO study suggests that offspring of pregnancies complicated by GDM have higher adiposity and impaired glucose metabolism at 10-14 years of age (66). Treatment of GDM (lifestyle interventions, metformin and insulin) has been shown to improve maternal and offspring perinatal outcomes (67) but fails to prevent childhood obesity (68). The exact mechanism leading to future obesity in cases of treated GDM remains unclear.

In our study, we assessed the effect of GDM on infant body composition up to 5 months of age. We diagnosed GDM based on NICE criteria (69) following OGTT at 28 weeks of pregnancy. Women diagnosed with GDM received regular antenatal care, lifestyle advice and treatment with metformin and/or insulin. Treatment interventions in the GDM group resulted in optimal glycaemic control, as indicated by the levels of HbA1c (mean value: 34.9 mmol/mol). We observed that GDM group gained less weight (mean weight gain was 3 kgr) than IOM recommendations (15) and was delivered ten days earlier than the control group of nGDM pregnancies. Using an accurate technique of measuring body composition (70), PEAPOD, we assessed infants at three time points: birth, 4 weeks and 5 months postpartum. We used statistically robust indexes (FMI, FFM) to allow adjustments for infant size. We presented data showing that treatment of GDM prevents neonatal macrosomia at the cost of reduced FFM. After adjusting for maternal characteristics we showed that reduced GWG and earlier induction of labour were the main determinants of low FFM in the GDM group. Finally, we demonstrated that by 5 months of age offspring of the GDM group, despite optimal maternal glycaemic control, develop accelerated growth driven by FM accretion.

Our study supported the observation of early adiposity rebound shown by Logan et al (71) in GDM offspring despite optimal maternal glycaemic control and exclusive breast feeding. Using paired MRI scans at 11 days and 10 weeks of post partum, they revealed that offspring exposed to maternal GDM have increased adiposity at 10 weeks despite similar adiposity levels at 11 days after delivery. Authors suggested that this observation may be due to altered hypothalamic signaling leading to hyperphagia or differences in the composition of breast milk. They did recognize though that data on breast milk composition in mothers with GDM are scarce and they suggested future research on this field. Our study not only supported this observation using another objective measure of adiposity but extend this observation to 5 months of postpartum in a much larger sample size.

The role of maternal hyperglycaemia in adverse metabolic programming is beyond doubt. The “Pedersen hypothesis” (72) describes that fetal exposure to excessive maternal

glucose leads to fetal hyperinsulinaemia, macrosomia and adiposity. We have previously presented data that maternal hyperglycaemia in early pregnancy is related to altered placental growth and epigenetic modifications which in turn result to altered tissue structure and function. Of great importance, maternal hyperglycaemia leads to lower leptin gene methylation in fetal adipocytes, resulting in higher cord blood leptin levels (Figure 7.9) (73). Recent ultrasound findings suggest that adverse fetal growth is present even at 20 weeks of gestation, preceding the diagnosis of GDM (74-76). Consistent with these findings, we showed that fetuses whose mothers will eventually develop GDM have higher estimated fetal weight and head circumference during the 20 weeks scan. In this study, we showed that despite optimal glycaemic control, offspring of pregnancies complicated by GDM have higher insulin resistance at birth, indicated by higher c-peptide levels for a given FM. The above observations suggest that adverse metabolic trajectories may be set prior to the diagnosis of GDM by causing some form of beta-cell dysfunction and optimization of intrauterine environment during early pregnancy is crucial to prevent future obesity.

We believe that the effect of developmental programming on offspring phenotype is unlikely to present during infancy, therefore we suggest that early adiposity rebound cannot be due to adverse hypothalamic programming, as proposed by Logan et al. Results of our systematic review and analysis of cord blood biomarkers in our cohort support the above statement. Cord blood leptin seems to be negatively related to adiposity up to 3 years of age, shifting to a positive correlation thereafter. Instead, we propose that the increased adiposity noted in early infancy in our GDM group is driven by low FFM at birth, a phenomenon known as “collateral fattening” (20). FFM is the most metabolically active tissue in our body and is responsible for 70% of Resting BMR. Data suggests a feedback system between FFM and satiety center. In cases of FFM excess hunger is increased to match the requirements (passive role) and in cases of FFM deficiency hunger is triggered to restore the deficit (77). “Collateral fattening” describes the excessive fat mass accretion as a result of body’s attempt to correct FFM deficit through hyperphagia. The concept was first described following the Minnesota Experiment at the end of World War II (78). Healthy adults after 24 weeks of semi-starvation followed by 20 weeks of re-feeding regained more weight and FM than they initially lost. Hyperphagia and fat mass accretion continued until FFM was completely restored (Figure 3.23). The imbalance in FM/FFM partitioning could be explained as an adaptive mechanism in which body stores higher amounts of FM in order to be prepared for the next nutritional scarcity. Furthermore, hyperphagia may exceed the synthetic capacity of FFM causing the excess calories to be deposited as FM. In summary, inadequate GWG and earlier induction of labor during GDM treatment prevent neonatal

macrosomia at the cost of FFM. The FFM deficiency triggers a “reactive mechanism” which results in “fat overshoot”. Rapid postnatal weight and FM accretion contribute further to the adverse metabolic programming and the development of future obesity.

7.9 Summary of the key messages

Diagnostic criteria and therapeutic approach of pregnancies complicated by GDM aimed to reduce perinatal outcomes and identify women at risk for type 2 diabetes mellitus. HAPO study revealed a continuous association between maternal glucose, adverse perinatal outcomes and neonatal adiposity without any clear “cut off” (21). Latest results, highlighting the association between GDM and childhood adverse metabolism, add further to the need of amending our approach in order to reduce the risk of future obesity for the offspring (66). In other words, management of GDM, from preconception to diagnosis and treatment, should be tailored not only to prevent short term but also long term adverse outcomes. Accumulating data suggests that we may interfere too late, when metabolic trajectories are already set by the effect of impaired glucose metabolism (namely insulin resistance or impaired glucose tolerance or hyperglycaemia) during the early stages of life (79). Ideally, we would like all mothers to have optimal metabolic profile in early pregnancy, therefore, we should implement lifestyle interventions in the preconception period. As a big percentage of pregnancies are unplanned and women do not see their doctors until the end of the first trimester it would be difficult to identify potential candidates (80). Considering the difficulties of engaging mothers during preconception or early pregnancy, researchers have turned their attention in creating risk models (using maternal characteristics and biomarkers) to identify high risk mothers during the first trimester. Results of ongoing studies (PRiDE and STRiDE) are highly anticipated as they will allow selective and targeted approach to those most needing it. On the other hand, raising public awareness on the importance of preconception care could potentially lead more women to seek medical advice. A pregnancy complicated by GDM, should not be considered as a catastrophic event for maternal and offspring metabolism but as a chance to improve maternal health for consequent pregnancies. Therefore, lifestyle interventions and follow up care should be implemented in the immediate postnatal period.

The term “Health Technologies” describes every intervention aiming to promote public health by preventing and treating diseases. Health Technology Assessment” assesses the cost-effectiveness and the impact of every implemented intervention. As such, randomized controlled trials have previously shown that GDM treatment improves

perinatal outcomes (e.g caesarian section) via controlling fetal growth (81). However, real life data suggests that induction of labor and rate of caesarian sections are higher in pregnancies complicated by GDM (74). Our results are consistent with these findings with our GDM group delivered on average 9 days earlier. Furthermore, we showed that intensified management of GDM led to weight gain below IOM recommendations. Early induction of labor and inadequate weight gain in our cohort was associated with reduced FFM, potentially causing early adiposity rebound and high cardiovascular risk in the future. Our findings are in agreement with data presented by Catalano et al (18) and suggest that “weight reduction” should be replaced by “weight gain limitation”. We propose that GWG targets should comply with IOM recommendations until new robust data is available. We hope that our data provides insight on the optimal timing of delivery and raise queries regarding current practices. New studies (Big Baby) looking at the association between time of delivery, short (still birth) and long (low FFM driving future obesity) term adverse outcomes will enable RCOG and NICE to develop evidence based guidance to direct clinical practice. The cost-effectiveness approach which is currently adopted by NICE shouldn't be based solely on immediate perinatal outcomes but also take into account long term adverse outcomes and the impact of childhood obesity on NHS resources.

7.10 Does one size fit all? Effect of ethnicity on body composition

There has been a long standing controversy regarding the optimal way to monitor fetal growth. Despite the universal agreement regarding customized growth charts based on gender and gestational age, there is no consensus regarding maternal ethnicity (82). The close relationship between rates of SGA and perinatal mortality seen when ethnic-specific standards are used has led many to support that growth charts should be also customized for maternal ethnicity (83). On the other hand, Intergrowth 21st study has recently shown that growth is affected more by health, socioeconomic and environmental factors rather than ethnic and genetic characteristics. The team suggested that variability in fetal/neonatal characteristics is <3% between ethnic groups, provided that maternal health and fetal intrauterine conditions are optimal (84). If we take a closer look at both approaches, we will realize that they convey the same message. Ethnic-specific standards do not take into account intrauterine environment and heavily rely on genetics. For example, a mother of SA origin born in Asia will come under the same category with a mother of SA origin born in a European country (first or second generation migrant). On the other hand, participants of the Intergrowth study had to fulfill very strict inclusion criteria in order to form a very low risk group which ensures optimal growth conditions for

the fetus. The “gap” between developed and developing countries, rural and urban areas sets the generalizability of these results under question. Overall, combined messages from both approaches reveal that fetal/ neonatal characteristics should be similar, provided that maternal characteristics and intrauterine environment remain constant.

Our study provides unique data on body composition of infants of SA and white European descent as we revealed no between group differences up to 5 months of age. Our data strongly suggests that the role of intrauterine programming is far more significant than this of genetic predisposition. In our cohort, both groups shared same maternal characteristics, except lower BMI in the SA cohort, which could potentially explain the lower birthweight observed in their offspring. We further examined surrogate markers of maternal metabolism and we remarkably showed that both groups shared similar insulin resistance and adipocytokines levels. Based on maternal demographics, we speculate that the improved metabolic and antenatal health of our SA cohort compared with SA residing in Asia could be a result of higher socioeconomic status, higher education, balanced diet and better access to healthcare services. We also hypothesize, that due to the “healthy migrant effect” mothers of SA origin experienced a favorable intrauterine environment as fetuses, resulting in higher height, higher weight and improved metabolic status (85). As a result, exposures and environments experienced by one generation (SA mothers) relate to the health development of the next generation (SA offspring) and the convergence of adiposity rates similar to native (White British) population.

In conclusion, we support that maternal intrauterine environment, irrespective of maternal ethnic background, can set offspring metabolic trajectories. Imprinted genes can be modified by antenatal fetal exposures and favorable intrauterine environment can affect structural and functional development of organs responsible for future metabolic health. Although of reasonable size (and the largest for having serial objective adiposity measurements until 5 months of age), the size of our cohort is one of the main limitations in the generalizability of our results. However, the use of detailed maternal demographics, maternal biomarkers and the assessment of infant composition with PEAPOD add to the validity and credibility of our results. It would be of great importance to follow up our cohort and assess metabolic markers in order to examine whether these findings are sustained in early childhood.

7.11 Conclusion – The intergenerational effect

Intergenerational effect can be summarized as “those factors and conditions experienced by one generation that relate to the development and health of subsequent generations”.

Intergenerational programming is a combination of three mechanisms:

- i) shared genes between mother and the offspring;
- ii) environmental conditions which may be similar across different generations and
- iii) intrauterine programming.

The latter mechanism describes the sequence of female fetuses exposed to adverse intrauterine environment becoming mothers with adverse metabolism and subsequently giving birth to offspring with impaired metabolic trajectories (Figure 7.9). We have previously described the role of intrauterine programming and provided evidence to suggest that what appears to be genetic may in fact be a programming influence that has been running through several generations. From conception to early postnatal life the future of every cell, every tissue is dictated by “developmental programming”. Human’s physiology, in other words, is programmed by the intrauterine conditions the fetus is growing. Understanding the timing of tissue development is crucial to assess whether interventions can affect the metabolic function in the future.

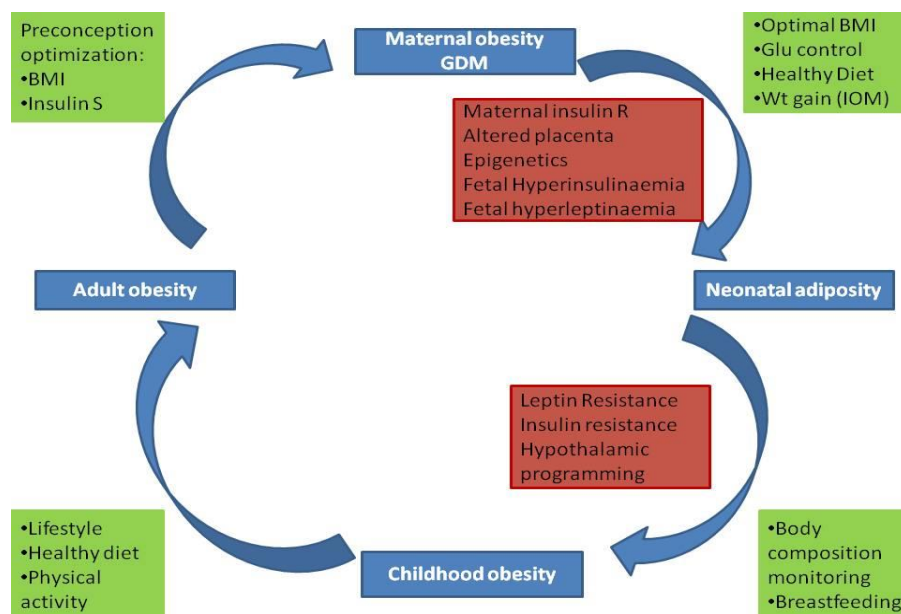


Figure 7.9: Developmental programming and intergenerational effect; Red boxes represent pathogenetic mechanisms; Green boxes represent intervention strategies. Insulin R: insulin resistance; Insulin S: insulin sensitivity

Pregnancy, from preconception to early postpartum, is the ideal opportunity to intervene as mothers are highly motivated to adopt behaviors which will protect their offspring. A temporary improvement in maternal health could potentially improve health of succeeding generations. Interventions, such as lifestyle changes, need to be implemented in the preconception period and continue throughout pregnancy. The development of risk assessment models to identify high risk pregnancies will allow targeted approach to those most in need, constituting a cost-effective way in tackling the obesity epidemic from its routes. The importance of early postpartum, a period of developmental plasticity, should not be ignored. For mothers, it should be an opportunity to improve their metabolic health and well being as they will be getting ready for subsequent pregnancies. For offspring, the potential use of early life biomarkers to identify those at high risk of future obesity, will allow implementation of early intervention strategies, such as parental education on the benefits of exclusive breastfeeding, healthy diet options and physical activity.

7.12 Future plans and recommendations

The current thesis is part of a larger study taking place at GEH, aiming to recruit 3,000 participants by 2022. It would be of great interest to see if findings from our cohort are consistent with these of a larger population. Our data suggests that insulin resistance is the missing link between maternal adverse characteristics and neonatal adiposity. The observational design of our study can only establish associations, but not causality. To further support the theories of “insulin resistance” and “fuel teratogenesis” we suggest the analysis of additional maternal biomarkers, such as triglycerides, free fatty acids and amino acids, looking at their correlation with maternal insulin resistance and neonatal adiposity. We strongly suggest follow up studies of our cohort looking at offspring body composition and metabolic markers in early childhood. We encourage the use of objective measures to assess body composition, in specific the child/adult version of PEAPOD, BODPOD. Data obtained could potentially shed light on the role of intrauterine programming on long term offspring metabolic trajectories and the predictive value of cord blood biomarkers on future obesity.

We presented unique data supporting that intrauterine exposures can override genetic predisposition. Specifically, we showed that offspring of SA origin born in the UK share similar infant body composition with White Europeans when maternal characteristics and intrauterine exposures are matched. In order to provide evidence on the generalizability of our findings, these associations need to be examined in larger cohorts. We further

suggest that ethnicity questionnaires should also capture information on the paternal ethnicity in an attempt to better characterize offspring's ethnic background. Fathers could potentially have an indirect epigenetic effect on offspring by influencing their partner's diet, thus influencing intrauterine environment.

Finally, we speculate that the timing of intervention strategies is crucial in the prevention of adverse outcomes. Therefore, we provide the following recommendations for future research and clinical practice:

- i) design of randomized control trials looking at the association of preconception interventions with maternal metabolism and objectively measured offspring adiposity;
- ii) creation of early pregnancy individualized risk models to identify pregnancies at high risk of developing GDM or adverse perinatal outcomes and
- iii) development of postnatal strategies aiming to improve maternal health for subsequent pregnancies to potentially reverse adverse intrauterine programming for the offspring as preconception interventions.

7.13 References

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Ethical considerations

Consent

Throughout our study, all participants had to sign a written consent form prior to being recruited. This consent form explicitly asked for the mothers' permission to carry out genetic and molecular tests on the blood and tissue samples (maintaining anonymity), even after the completion of the study, for medical and research purposes in the future. Research staff was always present during consenting in order to answer any questions or clarify further any statements on the form.

Only competent pregnant mothers with capacity to understand information about the study and give their valid consent were recruited. Due to the ethnic diversity of the study group we came across participants who did not have a good understanding of the English language. As we highly value the contribution of ethnic minorities in our study we made provisions for participants to understand information provided and give informed consent. In such cases, a qualified interpreter went through the patient information sheet and assisted in the informed consent process. Participants were also offered the choice of interpreter's presence in the subsequent study visits. During the study, we did not identify any participant with other communication barriers, such as reading or hearing difficulties.

Human Biological Material

Consent form clearly asked participants consent on collecting and storing serum and cord blood samples for further analysis, including gene tests. It also firmly stated that results would be kept confidential and not revealed to the individual. Purpose of storing samples was to analyze further biomarkers during the course of the study, on top of the initial biochemical tests, in an attempt to understand the underlying mechanisms and pathophysiology of GDM.

All samples were stored in the pathology laboratories at GEH under the local pathology network and under the custody of Prof. Saravanan (Chief Investigator). Only laboratory and research staff assigned to carry out investigations from the blood samples was given access to them.

Risks and Burdens

As this was a non-interventional study, the risks to the participants were minimal. All of the study visits coincided with participants' routine pregnancy visits with midwives or hospital clinics. However, some participants may have been inconvenienced by the two extra visits (4 weeks and 5 months postpartum), therefore we tried to arrange a suitable day and time for them, reimbursing any extra costs like transport or parking. Participants would usually spend 15 additional minutes during their recruitment visit in completing questionnaires for the study. However, they were asked to complete them during their two-hour wait for the repeat OGTT test.

Maternal blood sampling also coincided with routine blood testing (OGTT) and samples at birth were obtained from the cord and not from the mother. Anthropometric data collection was painless, aside from the use of calipers which was felt as "a pinch of the skin". ADP principles (utilizing circulating air and body's volume) ensured the safety of the PEAPOD measurement.

Non-research staff was asked to collect and centrifuge cord blood samples when participants delivered out of working hours. In order to thank them for their kind participation and reimburse them for the extra time spent in collecting samples for our study, a £5 voucher was provided for every ten cord blood samples collected. Furthermore, souvenirs, like pens, mugs and bags with the study logo, were provided to all staff in the maternity unit.

Benefits

As this was an observational study, there were no direct benefits for the participants. Knowing that their participation in the study would help raise awareness on the effect of maternal adverse risk factors on offspring's body fat mass and future health was on its own rewarding for the majority of participants. Furthermore, instant access to the body composition results of their child after the PEAPOD measurement was another important motivation.

Confidentiality

Recommendations from the NHS Code of Confidentiality and University of Warwick data protection act were strictly adhered to at all stages of the study. Upon recruitment, each

participant was given a unique study ID which followed them throughout the study. Data and bloods obtained were labeled with that ID number. Only the research team members who came into direct contact with the participants had access to their personal data, whilst everyone else had only access to their pseudoanonymised data via the unique study ID. The former group included the author and the two research midwives who had to take informed consent and carry out anthropometric measurements. Since this was a prospective longitudinal study, following up the participants throughout the pregnancy and immediate postpartum was an essential part of it. This is the reason why author and research midwives had access to the participants' obstetric records for the duration of the study.

Paper case report forms and files were stored in the Diabetes Research office. Access to the office was through a password protected door and allowed only to authorised members of the staff. After completion of the study all paper data was converted to electronic using password protected computers at GEH or University of Warwick premises. PEAPOD database was transferred to these computers using encrypted (password protected) USB sticks provided by the university or the hospital. Personal identifiable data was not required for the analysis, thus not entered to the electronic database.

Conflicts of Interest

As part of the Diabetes team at GEH, author is participating in the combined antenatal clinic for mothers with GDM therefore there may have been a conflict of interest. On the other hand, mothers with GDM was only a proportion of our study group. As our study was non-interventional, patients care and therapeutic plan was left to the discretion of the normal physician. From our perspective, we ensured that mothers who developed GDM received optimal care in our antenatal clinic, irrespective of their participation in the study.

Funding

Grants for the study have been secured by Medical Research Council (MRC), National Institute for Health Research (NIHR), Warwickshire Private Hospital Trust, George Eliot Hospital Diabetes Research fund and University of Warwick Alumni funds.

Sponsors

University of Warwick acted as the main sponsor of the study. As this was a clinical observational study involving pregnant patients, George Eliot Hospital (GEH) acted as the cosponsor for the clinical negligence aspect of the study.

GEH acted as the main cosponsor of the study and the co-employer of the author (honorary contract) and the academic supervisor/chief investigator, Prof. P.Saravanan. GEH was responsible for all the study related clinical procedures, maintaining standard operating procedures, updating protocols, training of staff, maintaining study related documents in safe areas, maintain site file as per Good Clinical Practice (GCP) guidelines and make all the above available for audit and inspection as necessary.

University of Warwick ensured all aspects of research governance was followed and provided GEH necessary support in any of the above aspects if required. University of Warwick provided GEH with all the relevant documents including the co-sponsorship agreement, indemnity cover (insurance document – yearly update) and ensured all the research staff who accessed the GEH facilities had all the up to date research training.

Appendices

An Evaluation of the Pea Pod System for Assessing Body Composition of Moderately Premature Infants; Forsum E, Olhager E and Törnqvist C - <i>Nutrients</i> 2016, 8(4), 238
Evaluation of air-displacement plethysmography for body composition assessment in preterm infants; Roggero P, Gianni ML, Amato O, Piemontese P, Mornioli D, Wong WW, Mosca F. <i>Pediatr Res.</i> 2012 Sep;72(3):316-20. doi: 10.1038/pr.2012.75. Epub 2012 Jun 5
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A new air displacement plethysmograph for the measurement of body composition in infants; Urlando A, Dempster P, Aitkens S. <i>Pediatr Res.</i> 2003 Mar;53(3):486-92
Evaluation of a new pediatric air-displacement plethysmograph for body-composition assessment by means of chemical analysis of bovine tissue phantoms; Sainz RD, Urlando A. <i>Am J Clin Nutr.</i> 2003 Feb;77(2):364-70
Preliminary evaluation of a new pediatric air displacement plethysmograph for body composition assessment in infants; Yao M, Nommsen-Rivers L, Dewey K, Urlando A. <i>Acta Diabetol.</i> 2003 Oct;40 Suppl 1:S55-8

Appendix 1: ADP validation against reference studies

PROSPERO International prospective register of systematic reviews

Review title and timescale

- 1 **Review title**
Give the working title of the review. This must be in English. Ideally it should state succinctly the interventions or exposures being reviewed and the associated health or social problem being addressed in the review.
Do cord blood adipocytokines predict body composition in infants and children up to 5 years of age?
- 2 **Original language title**
For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.
Do cord blood adipocytokines predict body composition in infants and children up to 5 years of age?
- 3 **Anticipated or actual start date**
Give the date when the systematic review commenced, or is expected to commence.
10/06/2017
- 4 **Anticipated completion date**
Give the date by which the review is expected to be completed.
30/09/2017
- 5 **Stage of review at time of this submission**
Indicate the stage of progress of the review by ticking the relevant boxes. Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. This field should be updated when any amendments are made to a published record.

The review has not yet started ☒

Review stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

Review team details

- 6 **Named contact**
The named contact acts as the guarantor for the accuracy of the information presented in the register record.
Christos Bagias
- 7 **Named contact email**
Enter the electronic mail address of the named contact.
c.bagias@warwick.ac.uk
- 8 **Named contact address**
Enter the full postal address for the named contact.
George Eliot Hospital, College street, Nuneaton, CV10 7DJ
- 9 **Named contact phone number**
Enter the telephone number for the named contact, including international dialing code.
00447813622134
- 10 **Organisational affiliation of the review**
Full title of the organisational affiliations for this review, and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

Appendix 2: Prospero registration

https://ovidsp.tx.ovid.com/sp-3.27.0b/ovidweb.cgi Ovid: Search Form

Ovid® Wolters Kluwer
My Account Ask A Librarian Support & Training Help Logged in as CHRISTOS BAGIAS at WARWICK UNIVERSITY Logoff

Search Journals Books Multimedia My Workspace Mobile

▼ Search History (9) View Saved

#	Searches	Results	Type	Actions	Annotations
1	Leptin/ or Adiponectin/	64928	Advanced	Display Results More ▾	Contract
2	Fetal Blood/	8318	Advanced	Display Results More ▾	
3	exp Adiposity/ or exp Obesity/	433560	Advanced	Display Results More ▾	
4	body composition/	50149	Advanced	Display Results More ▾	
5	fetal growth.mp. or fetus growth/	23044	Advanced	Display Results More ▾	
6	anthropometry.mp. or anthropometry/	52625	Advanced	Display Results More ▾	
7	1 and 2	52	Advanced	Display Results More ▾	
8	3 or 4 or 5 or 6	524180	Advanced	Display Results More ▾	
9	7 and 8	15	Advanced	Display Results More ▾	

Save Remove Combine with: AND OR

Save All Edit Create RSS View Saved

Basic Search | Find Citation | Search Tools | Search Fields | **Advanced Search** | Multi-Field Search

1 Resource selected | Hide | Change

Embase 2013 to 2017 Week 43

Enter keyword or phrase (* or \$ for truncation)

Keyword Author Title Journal Search

▼ Limits (close) Include Multimedia Map Term to Subject Heading

☐ Full Text ☐ Latest Update ☐ Abstracts
☐ Human ☐ Cochrane Library ☐ English Language

Publication Year - -

Status NLM Status

Article-in-Press Status
 Conference Abstract Status
 In-Data-Review
 In-Process
 MEDLINE

Appendix 3: Literature search on EMBASE



Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies

Criteria	Yes	No	Other (CD, NR, NA)*
1. Was the research question or objective in this paper clearly stated?			
2. Was the study population clearly specified and defined?			
3. Was the participation rate of eligible persons at least 50%?			
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?			
5. Was a sample size justification, power description, or variance and effect estimates provided?			
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?			
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?			
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?			
10. Was the exposure(s) assessed more than once over time?			
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?			
12. Were the outcome assessors blinded to the exposure status of participants?			
13. Was loss to follow-up after baseline 20% or less?			
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?			

Quality Rating (Good, Fair, or Poor) (see guidance)
Rater #1 Initials:
Rater #2 Initials:
Additional Comments (If POOR, please state why):

*CD, cannot determine; NA, not applicable; NR, not reported

Guidance for Assessing the Quality of Observational Cohort and Cross-Sectional Studies

The guidance document below is organized by question number from the tool for quality assessment of observational cohort and cross-sectional studies.

Question 1. Research question

Did the authors describe their goal in conducting this research? Is it easy to understand what they were looking to find? This issue is important for any scientific paper of any type. Higher quality scientific research explicitly defines a research question.

Questions 2 and 3. Study population

Did the authors describe the group of people from which the study participants were selected or recruited, using demographics, location, and time period? If you were to conduct this study again, would you know who to recruit, from where, and from what time period? Is the cohort population free of the outcomes of interest at the time they were recruited?

An example would be men over 40 years old with type 2 diabetes who began seeking medical care at Phoenix Good Samaritan Hospital between January 1, 1990 and December 31, 1994. In this example, the population is clearly described as: (1) who (men over 40 years old with type 2 diabetes); (2) where (Phoenix Good Samaritan Hospital); and (3) when (between January 1, 1990 and December 31, 1994). Another example is women ages 34 to 59 years of age in 1980 who were in the nursing profession and had no known coronary disease, stroke, cancer, hypercholesterolemia, or diabetes, and were recruited from the 11 most populous States, with contact information obtained from State nursing boards.

In cohort studies, it is crucial that the population at baseline is free of the outcome of interest. For example, the nurses' population above would be an appropriate group in which to study incident coronary disease. This information is usually found either in descriptions of population recruitment, definitions of variables, or inclusion/exclusion criteria.

You may need to look at prior papers on methods in order to make the assessment for this question. Those papers are usually in the reference list.

If fewer than 50% of eligible persons participated in the study, then there is concern that the study population does not adequately represent the target population. This increases the risk of bias.

Question 4. Groups recruited from the same population and uniform eligibility criteria

Were the inclusion and exclusion criteria developed prior to recruitment or selection of the study population? Were the same underlying criteria used for all of the subjects involved? This issue is related to the description of the study population, above, and you may find the information for both of these questions in the same section of the paper.

Appendix 4: NIH quality assessment tool

	Meyer (104)	Meyer (105)	Schneider (103)	Kadakia (92)	Karakosta (97)	Chaoimh (91)	Donelly (85)	Teague (88)	Josefson (89)	Brunner (9)
Research question or objective clearly stated?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Study population clearly specified and defined?	Y	Y	Y	N	Y	Y	Y	Y	Y	Y
Participation rate of eligible persons $\geq 50\%$?	N	Y	Y	Y	N	Y	Y	Y	Y	Y
Subjects from same or similar populations; Inclusion and exclusion criteria applied uniformly to all participants	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Sample size justification, power	N	N	N	N	N	N	N	N	Y	N
Exposure(s) of interest measured prior to the outcome(s) being measured?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Sufficient timeframe to see an association between exposure and outcome	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Examination of different levels of the exposure as related to the outcome	Y	Y	Y	Y	Y	Y	NA	NA	Y	Y
Independent variable clearly defined	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Exposure(s) assessed more than once over time?	NA	Y	NA	N	Y	Y	NA	NA	N	Y
Dependent variables clearly defined	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Outcome assessors blinded to the exposure status of participants?	N	N	N	N	N	N	N	N	N	N
Loss to follow-up $\leq 20\%$	N	N	Y	NA	Y	N	NA	N	N	Y
Key potential confounders measured and adjusted for their impact	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
Overall	i	i	i	ii	ii	ii	ii	ii	ii	ii

	Boeke (43)	Simon (94)	Basu (95)	Mantzoros (42)	Inami (96)	Valuniene (90)	Martinez (78)	Javaid (87)	Tsai (102)	Lindsay (77)	Geary (106)	Clapp (93)
Research question or objective clearly stated?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Study population clearly specified and defined?	Y	Y	N	Y	Y	N	N	N	Y	Y	Y	N
Participation rate of eligible persons $\geq 50\%$?	N	N	NA	N	Y	Y	NA	Y	Y	N	Y	N
Subjects from same or similar populations; Inclusion and exclusion criteria applied uniformly to all participants	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y
Sample size justification, power	N	N	N	N	N	N	N	N	N	N	N	N
Exposure(s) of interest measured prior to the outcome(s) being measured?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Sufficient timeframe to see an association between exposure and outcome	Y	Y	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y
Examination of different levels of the exposure as related to the outcome	Y	NA	Y	Y	NA	NA	NA	NA	N	NA	NA	NA
Independent variable clearly defined	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Exposure(s) assessed more than once over time?	Y	Y	NA	Y	Y	NA	NA	Y	NA	NA	NA	NA
Dependent variables clearly defined	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Outcome assessors blinded to the exposure status of participants?	N	N	N	N	N	N	N	Y	N	N	N	N
Loss to follow-up $\leq 20\%$	Y	N	NA	Y	Y	N	NA	NA	NA	NA	NA	NA
Key potential confounders measured and adjusted for their impact	Y	Y	Y	Y	N	Y	N	Y	Y	N	Y	Y
Overall	ii	ii	i	ii	i	i	i	ii	ii	i	ii	i

Appendix 5: Quality assessment score of the included studies; Y: yes, N: no, NA: not applicable; Studies are rated 0 for poor, i for fair, ii for good



GEORGE ELIOT
HOSPITAL
NHS TRUST

CONTRACT FOR PLACEMENT

NAME: **Dr Christos Bagias**

DATE: **22/03/2016**

ADDRESS: 30 Granville Street, Apartment 115, Cutlass
Court, Birmingham, B1 2LJ

This is a Contract for Placement not a Contract of Employment between the George Eliot Hospital NHS Trust (the Trust) and yourself to enable you to work within the George Eliot Hospital NHS Trust. Whilst on placement to the Trust, you are required to comply with its policies and procedures.

RESPONSIBLE TO: Dr Ponnusamy Saravanan

DETAILS OF PLACEMENT: Warwick Medical School

START DATE: 7th March 2016

1. Accountable when undertaking Functions under this Contract:

It is the Trust intention that any oral instruction or written descriptions of your required duties and responsibilities should serve as a guide to the major areas for which you will be responsible.

2. Hours of Duty:

Your hours of duty should be agreed between yourself and your Supervising Consultant or Manager, but will be those necessary for the proper and efficient performance of your duties.

3. Indemnity Arrangements

Appendix 6: Author's honorary contract with George Eliot Hospital (GEH)

Dear George Eliot/ Warwick University Sponsor Representative,

RE: 214062 - Role of maternal adverse/risk outcomes on offspring's body composition (PEAPOD)

This email confirms that George Eliot Hospital NHS Trust has the capacity and capability to deliver the above referenced study. Please find attached our agreed Statement of Activities as confirmation.

We agree to start this study and give the green light to begin.

Delivery teams – Please note that you have now been added to the study record on EDGE, the Trust's local information management system used to record all research at the Trust, including real time recruitment input by the clinical delivery teams. EDGE can be accessed at www.EDGE.nhs.uk

Please refer to HRA Approval letter dated 05/01/2017 for latest versions of approved documentation.

If you wish to discuss further, please do not hesitate to contact me.

Kind regards

Shelley

Shelley Grant

Study Support Facilitator | CRN: West Midlands | NIHR Clinical Research Network (CRN)

Appendix 7: Local R&D approval letter

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

31 October 2016

Dr PONNUSAMY SARAIVANAN
Warwick Medical School (UHCW Campus)
University of Warwick
CV2 2DX

Dear Dr SARAIVANAN

Study title:	Role of maternal adverse (risk) outcomes and ethnicity on offspring body fat content in White Caucasians and South Asians
REC reference:	16/NW/0796
Protocol number:	N/A
IRAS project ID:	214062

Thank you for responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved by the sub-committee.

Appendix 8: REC approval

Dr Ponnusamy Saravan
Warwick Medical School (UHCW Campus)
University of Warwick
CV2 2DX

Email: hra.approval@nhs.net

05 January 2017

Dear Dr Saravan

Letter of HRA Approval

Study title:	Role of maternal adverse (risk) outcomes and ethnicity on offspring body fat content in White Caucasians and South Asians
IRAS project ID:	214062
REC reference:	16/NW/0796
Co-Sponsors	University of Warwick and George Eliot Hospital NHS Trust

I am pleased to confirm that HRA Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Appendix 9: HRA approval

Site Signature and Delegation Log



Site Name: _____ Location: _____

Please use the 'Trial Responsibilities Key' below to indicate which responsibilities have been agreed locally. Only members of the research team who are authorised by the Principal Investigator or Trial Coordinating Centre are permitted to undertake the trial responsibilities.

Should any member of the research team who has been delegated responsibilities below either join or leave post, an updated Site Signature and Delegation Log must be sent to the Diabetes research team Office immediately, to indicate who the responsibility has now been transferred to.

Trial Responsibility Key: (add any additional responsibilities as appropriate)

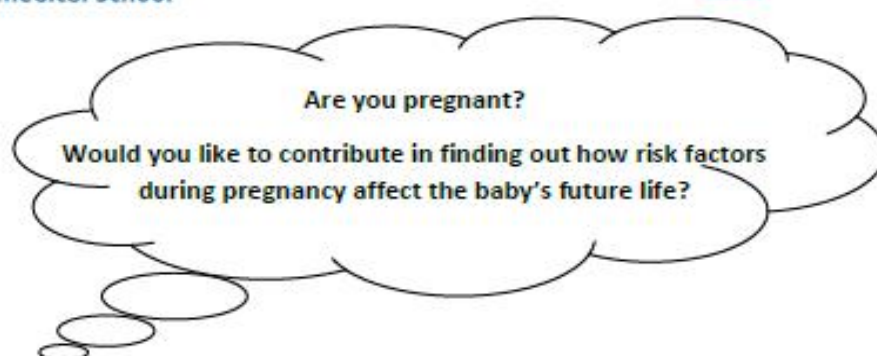
1	Overall responsibility for the trial at the site ^a	9	Sample collection (e.g. blood, urine) ^c
2	Explain trial to participants ^c		
3	Informed consent procedure ^c		
4	Sign informed consent form ^b		
5	Data entry into CRF's ^c		
6	SAE reporting to sponsor ^b		
7	Investigator site file maintenance ^c		
8	Measurements (e.g. blood pressure, height, weight) ^c		

Key: a = P.I. only
b = medically qualified personnel
c = suitably trained personnel e.g. research nurse, physiotherapist.

I confirm that I take overall responsibility for the conduct of this study at the above site, and that the trial personnel listed below are authorised to perform trial responsibilities on my behalf as indicated, within the dates indicated. I confirm that they agree to take on these responsibilities and are qualified and appropriately informed about the trial.

Name of Principal Investigator: _____ Signature: _____ Initials: _____ Date: _____

Appendix 10: Study delegation logbook



We are conducting a new study at George Eliot Hospital to find out how diabetes during pregnancy and other metabolic factors (ethnicity, obesity, pre-eclampsia) affect baby's future risk of developing diabetes, increased weight and cardiovascular disease.

Using PEAPOD, an innovative machine which only exists in few NHS Trusts, we can measure your baby's body fat and muscle mass in less than 5 minutes.



If you are interested in participating, please contact our research team on 02476153592 or speak to your midwife and GP for more information.

Hope to hear from you!

Appendix 11: Study advertising poster

Participant information sheet –PEAPOD study

Many thanks for participating in our study. As you already know, soon after the delivery, we will be measuring baby's weight, length, size of the head and skin fold thickness. The skin fold thickness tells us a rough estimate of baby's fat content. To improve the accuracy of this estimate, we have managed to secure additional funding to purchase an equipment called PEAPOD funded by a charity and West Midlands research network. George Eliot Hospital is one of the very few NHS Trusts that has this machine which can perform these measurements. This machine gives us an accurate estimate of baby's body fat content as well as its distribution.

PEAPOD is a computerized measuring machine and the accuracy is not affected by most common baby behaviors such as crying and movement. The whole procedure should only take 3-7 minutes. This involves undressing the baby, weighing him/her in the electronic scale as well as the actual measurement inside the cot for 2-3 minutes. The cot has a window so you can see your baby throughout the procedure (see picture). Mothers tend to describe the procedure as their baby being in an aeroplane.



Is the procedure distressing to the baby?

These techniques have been used thousands of times and not proven to be distressing at all to the babies. They do not involve any exposure to radiation or noise. In the unlikely scenario that your baby becomes distressed, the procedure will be abandoned and a further attempt will be done only when appropriate i.e. the child is calm with consent from the parent.

Appendix 12: Participant information sheet (short version)

Consent form

Study Title: Role of maternal adverse (risk) outcomes and ethnicity on offspring body fat content in White Caucasians and South Asians

Centre Number:

Participant Identification Number:

Name of Researcher:

Please initial box

1. I confirm that I have read the Participant Information Sheet (Version 1.0) for the above study. I have had the opportunity to ask questions and have them answered satisfactorily
2. I understand that my participation is voluntary and that I am free to withdraw from the study at any time without my medical care being affected
3. I agree for blood and tissue samples to be taken from me and the placenta and umbilical cord after delivery. I understand that various tests will be carried out on these samples, including gene tests, but my identity will be kept strictly confidential and that the results will not be made available to me personally.
4. I give permission for relevant sections of my medical notes and data collected during the study to be looked at by authorised researchers, individuals from regulatory authorities or the NHS Trust, where it is relevant to the study
5. I agree for my General Practitioner and Obstetric team (including doctors and midwives) to be informed of my participation in this study
6. I agree to take part in the study

☐
☐
☐
☐
☐
☐

Name of Patient (Print)

Signature of Patient

Date

Name of Researcher

Signature of Researcher

Date

Appendix 13: Consent form

PEAPOD Study – Case Report Form

Visit 1 (Recruitment Visit, During OGTT or at Delivery)

Participant Name: _____ Date of birth: _____

Participant Study ID Number: _____

Date of Assessment: _____ Place of Assessment: _____

Part 1 – Study Recruitment

1. Inclusion / Exclusion criteria

Inclusion Criteria

- All participants **MUST** be 18 – 45 years of age Yes ☐ No ☐

Exclusion Criteria

Participant is not eligible for study if any of the following are present:

- Congenital abnormalities (if they are consented after delivery) Yes ☐ No ☐
- Pre-existing diabetes Yes ☐ No ☐

2. Risk factors

- High-risk group for GDM (at least 1 of the following risk factors) :
 - previous GDM Yes ☐ No ☐
 - previous unexplained still birth or baby >4.5kg Yes ☐ No ☐
 - first degree relative with diabetes Yes ☐ No ☐
 - ethnic minority group (South Asians, Middle-Eastern, Afro-Caribbean) Yes ☐ No ☐
- Diagnosis at current pregnancy:
 - GDM Yes ☐ No ☐
 - Pre-eclampsia Yes ☐ No ☐
 - BMI >30kg/m² Yes ☐ No ☐
 - SGA Yes ☐ No ☐

3. Consent form

A signed and dated informed consent form should be obtained from the participant prior to any study-related activity.

Copy of Participant Information Sheet and Consent Form given to participant Yes ☐ No ☐

Copy of Consent Form filed in research file Yes ☐ No ☐

Part 3 – Past Pregnancies

If this not your first baby, please record details of previous births (Page 5 of Green Maternity Notes)

	BW (g)	Gest age	Sex	Anomaly Y/N	C-section Y/N	IUGR Y/N	GDM Y/N
Baby 1							
Baby 2							
Baby 3							

Any previous miscarriages/ Stillbirths: _____

Part 4 – Mother's measurements

Booking BMI: _____

		Booking visit	OGTT
Height (without shoes) in cm			
Weight (in kg)			
Skinfold thickness (in cm_)	Triceps		
	Subscapular		
Waist circumference (in cm)*			
Systolic BP			
Diastolic BP			

* Measure on bare abdomen

Part 5 – Diagnosis of GDM

Current gestational age in days (at time of GTT/Visit 1): _____ EDD: ____/____/____

For completion subsequently

Has the participant been diagnosed with GDM? Yes ☐ No ☐

If yes, is it according to GTT results? Yes ☐ No ☐

Results of GTT (when available): Serum glucose at t = 0 mins _____ mmol/L

Serum glucose at t = 120 mins _____ mmol/L

If yes, what were the cut-offs used? _____

Part 6 – Laboratory tests

1. Blood tests

- a) Give a blood test form and enclosed blood bottles to the participant (labelled in bright yellow as 'Visit 1 Bloods') Yes ☐ No ☐
- b) Leave 6 'Study ID stickers' (pre-printed with Participants Study ID number) inside blood form

Yes ☐ No ☐

Urine sample collected

Yes ☐ No ☐

Part 7 – Questionnaires

Please give the mother the attached questionnaire pack to complete before she leaves Visit 1

Yes ☐ No ☐

Attach the completed questionnaires with the mother's consent form to be sent back to the Research Team

Yes ☐ No ☐

Part 8 – Visit sign-off

_____	_____	_____
Signature	Name	Job title

Date		

Appendix 14: CRF visit 1

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ days per week

☐ No vigorous physical activities → Skip to question 3

2. How much time did you usually spend doing vigorous physical activities on one of those days?

_____ hours per day

_____ minutes per day

☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ days per week

☐ No moderate physical activities → Skip to question 5

4. How much time did you usually spend doing moderate physical activities on one of those days?

_____ hours per day

_____ minutes per day

☐ Don't know/Not sure

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

_____ days per week

☐ No walking → Skip to question 7

6. How much time did you usually spend walking on one of those days?

_____ hours per day

_____ minutes per day

☐ Don't know/Not sure

The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the last 7 days, how much time did you spend sitting on a week day?

_____ hours per day

_____ minutes per day

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

EuroQol-5D Questionnaire

Section 1

Please indicate which statement describes your own health state today. Please answer all by placing a tick ☐ in one of the three options for each question.

Mobility

- ☐ I have no problems in walking about
- ☐ I have some problems in walking about
- ☐ I am confined to bed

Self-care

- ☐ I have no problems with self care
- ☐ I have some problems with washing or dressing myself
- ☐ I am unable to wash and dress myself

Usual activities

- ☐ I have no problem in performing my usual activities(e.g. work, study, housework, leisure activity)
- ☐ I have some problems in performing my usual activities
- ☐ I am unable to perform my usual activities

Pain/Discomfort

- ☐ I have no pain or discomfort
- ☐ I have moderate pain or discomfort
- ☐ I have extreme pain or discomfort

Anxiety/Depression

- ☐ I am not anxious or depressed
- ☐ I am moderately anxious or depressed
- ☐ I am extremely anxious or depressed

Section 2

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked by 100 and the worst state is marked by 0.

We would like you to indicate on this scale how good or bad your own health is, in your opinion. Please do this by drawing a single line from the box below to whichever point on the scale indicates how your health state is.

(BEST imaginable health state)

100

90

80

70

60

50

40

30

20

10

0

Your own
health today

(WORST imaginable health state)

0

Socio Economic Questionnaire

SECTION A – MARITAL STATUS

Q1) What is your legal marital or same-sex civil partnership status?

- a) Never married and never registered a same-sex civil partnership ☐
- b) Married ☐
- c) Separated, but still legally married ☐
- d) Divorced ☐
- e) Widowed ☐
- f) In a registered same-sex civil partnership ☐
- g) Separated, but still legally in a same-sex civil partnership ☐
- h) Formerly in a same-sex civil partnership which is now legally dissolved ☐
- i) Surviving partner from a same-sex civil partnership ☐

SECTION B – EDUCATION

Q2) Which of these qualifications do you have?

- a) 1 - 4 O levels / CSEs / GCSEs (any grades), Entry Level, Foundation Diploma ☐
- b) NVQ Level 1, Foundation GNVQ, Basic Skills ☐
- c) 5+ O levels (passes) / CSEs (grade 1) / GCSEs (grades A*- C), School Certificate, ☐
- d) 1 A level / 2 - 3 AS levels / VCEs, Higher Diploma ☐
- e) NVQ Level 2, Intermediate GNVQ, City and Guilds Craft, BTEC First / ☐
- f) General Diploma, RSA Diploma ☐
- g) Apprenticeship ☐
- h) 2+ A levels / VCEs, 4+ AS levels, Higher School Certificate, Progression / ☐
- i) Advanced Diploma ☐
- j) NVQ Level 3, Advanced GNVQ, City and Guilds Advanced Craft, ONC, ☐
- k) OND, BTEC National, RSA Advanced Diploma ☐
- l) Degree (for example BA, BSc), Higher degree (for example MA, PhD, PGCE) ☐
- m) NVQ Level 4 - 5, HNC, HND, RSA Higher Diploma, BTEC Higher Level ☐
- n) Professional qualifications (for example teaching, nursing, accountancy) ☐
- o) Other vocational / work-related qualifications ☐
- p) Foreign qualifications ☐
- q) No qualifications ☐

SECTION C - EMPLOYMENT

Q3) What is your employment status?

- a) Employed ☐
- b) Sheltered employment ☐
- c) Unemployed ☐
- d) Student ☐
- e) Housewife ☐
- f) Retired ☐
- g) Other ☐

Q4) If employed: How would you describe your occupational status?

Occupation type	Occupation Examples	Response
1. Higher managerial, administrative and professional occupations	Chief executives and senior officials, Managers and directors, Higher professional occupations, Nurses, Teaching professionals	
2. Intermediate occupations	Legal associate professionals, National/Local government administrative occupations, Pensions and insurance clerks and assistants, Personal assistants and other secretaries.	
3. Small employers and own account workers	Bricklayers and masons, Roofers, roof tilers and slaters, Child minders and related occupations, Market and street traders and assistants.	
4. Lower supervisory and technical occupations	Rail transport operatives, Senior care workers, Vehicle technicians, mechanics and electricians, Bakers and flour confectioners, Cleaning and housekeeping managers and supervisors	
5. Semi-routine and routine occupations	Receptionists, Upholsterers, Florists, Care workers and home carers, Hairdressers and barbers	
*Never worked and long-term unemployed		

SECTION D – HOUSEHOLD INCOME

Q5) From the options below, can you please select the group which represents your total household/family income from all sources (wages, salaries, tax credits and benefits) before deductions for income tax and National Insurance etc.

- i. Up to £5,199 ☐
- ii. £5,200 up to £10,399 ☐
- iii. £10,400 up to £15,599 ☐
- iv. £15,600 up to £20,799 ☐
- v. £20,800 up to £25,999 ☐
- vi. £26,000 up to £31,199 ☐
- vii. £31,200 up to £36,399 ☐
- viii. £36,400 up to £51,599 ☐
- ix. £52,000 and above ☐

Q6) How many people aged 14 and over are there in the household?

.....

Q7) How many people aged under 14 are there in the household?

.....

PHQ-9 Depression

	Not all	at Several days	More than half the days	Nearly every day
1. Little interest in doing things.....	0	1	2	3
2. Feeling down, depressed, or hopeless.....	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much.....	0	1	2	3
4. Feeling tired or having little energy.....	0	1	2	3
5. Poor appetite or overeating.....	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down.....	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television.....	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual.....	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way.....	0	1	2	3

Column totals + + +

= Total Score

Not difficult at all Somewhat difficult Very difficult Extremely difficult

Appendix 15: Study questionnaires

Visit 2 (Delivery)

Participant Study ID Number: _____ Place of assessment _____

Part 1 – Neonatal/Baby's details: (Yellow notes-page 20)

Is this a multiple pregnancy? Yes ☐ No ☐

If yes, state number of babies _____

	DOB	Sex	Gestational age (page 2)	Apgar Score			Congenital Anomaly Y / N	Still birth Y / N	ICU/SCBU Admission Y / N (see plans for transfer-p20)
				1	5	10			
Baby 1									
Baby 2									

Any other complications-----

Part 2 – Pregnancy History

1. Please list any medical or obstetric problems which have developed since the last visit (see Page 2)

Fetal growth restriction Yes ☐ No ☐

Antepartum haemorrhage Yes ☐ No ☐

Hypertension/Proteinuria Yes ☐ No ☐

Pre-eclampsia Yes ☐ No ☐

Other _____

2. Please list any other medication the participant took since the last visit

Part 3 – Delivery History

Normal / Induction/ Instrumental / Elective CS/ Emergency CS

If C-Section state indication- Fetal compromise/ Failure to progress/Ante partum haemorrhage/ Breech/Maternal request / Other _____ (page 16 of yellow notes)

Part 4 – Diagnosis of GDM

Has there been a diagnosis of GDM

Yes ☐ No ☐

If No, move to section 5

If yes, circle what treatment is being given: Insulin / Metformin / Diet / Other drugs _____

Last HbA1c recorded prior to delivery: _____

Part 5 –Anthropometry**a) Maternal anthropometry**

		1	2
Weight (in kg)			
Skinfold thickness (in cm_)	Triceps		
	Subscapular		
Systolic BP			
Diastolic BP			

b) Baby anthropometry

		Baby 1		Baby 2	
		1	2	1	2
Baby's birth weight (pg 20 of Yellow Notes)					
Head circumference					
Mid-arm circumference					
Chest circumference					
Abdominal circumference					
Skinfold thickness	Triceps				
	Subscapular				

c) PEAPOD

	Baby 1	Baby 2
% Fat		
% Fat free mass		
Fat mass (kg)		
Fat Free Mass (kg)		

Part 6 – Visit Sign-off

_____	_____	_____	_____
Signature	Date	Name	Job title

VISIT 3 CHECKLIST

- | | | | | |
|--|-----|--------------------------|----|--------------------------|
| • Participant happy to continue and Informed consent still valid | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| • Case Report Form (CRF) completed for Visit 2 | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| • Mother's weight and Baby's measurements recorded | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| • PEAPOD measurements completed | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| • Cord blood taken | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| • Placenta/ Cord tissue taken | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| • If CS – adipose samples taken | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |
| • Post natal GTT been booked (for GDM) or Visit 3 discussed with participant | Yes | <input type="checkbox"/> | No | <input type="checkbox"/> |

Appendix 16: CRF visit 2

Participant Study ID Number: _____

Date of Assessment: _____ Place of assessment _____

Part 1 – Post-partum period

Mother breast feeding? _____

Mother's current weight _____

Part 2 – Baby's details

Please list any medical problems for the baby since birth? _____

Baby anthropometry:

Mid-arm circumference					
Chest circumference					
Abdominal circumference					
Skinfold thickness	Triceps				
	Subscapular				
Length of the baby					

PEAPOD

	Baby 1	Baby 2
% Fat		
% Fat free mass		
Fat mass (kg)		
Fat Free Mass (kg)		

Part 3 – Questionnaires

Edinburgh wellbeing questionnaire (WEMWBS)	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>
Quality of Life and Socioeconomic	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>
Pittsburgh Sleep Quality Index	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>
Breast feeding questionnaire	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>

Part 4 – Visit sign-off

_____	_____	_____	_____
Name	Job title	Signature	Date

VISIT 3 CHECKLIST

• Participant happy to continue and Informed consent still valid	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>
• Case Report Form (CRF) completed for Visit 3	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>
• Mother's weight recorded	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>
• Baby's anthropometry and PEAPOD	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>
• Questionnaires completed	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>
• If GDM, GTT bloods collected	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>

Appendix 17: CRF visit 3 and 4

Breastfeeding Sub-study

Questionnaire

Participant Study ID Number: _____

Date of questionnaire: _____

Name of Researcher/Student completing questionnaire _____

Q1) Did you breastfeed your baby when he/she was born? **Yes / No**

If no move to question 4

Q2) Have you stopped breast feeding? **Yes / No**

If no move to question 4

Q3) If Yes how old was your baby when you stopped? _____

Q4) Did you use formula top-ups? **Yes / No**

If no move to question 6

Q5) How old was your baby when you started formula top ups? _____

Q6) How old was your baby when you weaned him/her (i.e. introduced solid foods)?

Q7) How do you classify your baby's feeding? (Circle the right answer)

Predominantly breast fed

Predominantly formula fed

Mixed fed

The Pittsburgh Sleep Quality Index (PSQI)

Instructions: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions. During the past month,

1. When have you usually gone to bed? _____
2. How long (in minutes) has it taken you to fall asleep each night? _____
3. When have you usually gotten up in the morning? _____
4. How many hours of actual sleep do you get at night? (This may be different than the number of hours you spend in bed) _____

5. During the past month, how often have you had trouble sleeping because you...	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f. Feel too cold				
g. Feel too hot				
h. Have bad dreams				
i. Have pain				
j. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)
9. During the past month, how would you rate your sleep quality overall?				

Appendix 18: Feeding and sleep quality questionnaires

Abstracts and Publications

Abstracts:

10th DOHaD world conference, Rotterdam, 2017

- Assessment of infant body composition using air displacement plethysmography
- Factors affecting placental weight
- Pregnant women of South Asian origin have higher prevalence of symptoms suggestive of anxiety and depression in early pregnancy
- GLP-1 profile during glucose tolerance test in gestational diabetes mellitus

6th International Nutrition and Growth conference, Valencia, 2019

- Cord blood adipocytokines and body composition up to 5 years of age: A systematic review

10th International Symposium on Diabetes, Hypertension, Metabolic Syndrome and Pregnancy, Florence, 2019

- Impact of gestational diabetes mellitus on infant body composition

International Diabetes Federation (IDF) annual conference, Korea, 2019

- Impact of gestational diabetes mellitus on infant body composition during the first 5 months of life
- Association between treatment modality in gestational diabetes mellitus and objectively measured neonatal adiposity
- First trimester HbA1c predicts objectively measured neonatal adiposity in pregnancies complicated by gestational diabetes mellitus

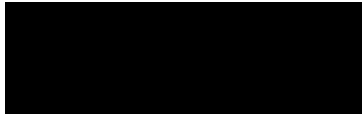
Publications:

- Cord blood leptin, adiponectin and body composition at birth and up to 5 years of age: A systematic review (pending peer review)
- Impact of gestational diabetes mellitus on infant body composition: A longitudinal, observational study (pending peer review)

Signed statement

I am aware of University regulations governing plagiarism and I declare that this document is all my own work except where I have stated otherwise. I consider myself ready to receive a PhD award.

Signed

A solid black rectangular box used to redact the signature of the student.

CHRISTOS BAGIAS

Date

17.10.2019